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Journal

Summary

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Picture on front page:
2021 Pezcoller Foundation – AACR International Award for Extraordinary Achievement in Cancer Research winner:
Dr. Hans Clevers

June 2021
Editorial

We are very pleased to be able to resume our Pezcoller Symposia this year, after the forced 2020 interruption, the first in 32 years.
This online modality was a mandatory choice, as for everyone. It represented however a big challenge for many reasons and especially for the need to match the oral presentations with the time differences of the various speakers, from California to Central Europe.
This modality on the other hand, has favored a large number of attendants from many countries, including non-European ones. This is the first time that we have had such a number of registered participants, 432, many of whom wouldn't otherwise have been able to attend in presence. Moreover, this large number is also a clear indicator of the great interest aroused by the topic of the symposium: **Aging and Cancer**.
We had also a good number of abstracts submitted for poster presentations at this symposium, and 24 of them were of outstanding or excellent quality and accepted for presentation.
Finally, last but not least, the editors of 4 important scientific journals (Nature, Nature Communications, Cell Press, Trends in Cancer) will attend the symposium.
So, this year symposium promises to be once again an exciting scientific event. We are deeply grateful to the chairman David Livingston and all the members of the standing committee, in particular Alberto Bardelli, Fabrizio D'Adda di Fagagna, Giannino Del Sal, Massimo Loda, Stefano Piccolo, Maria Rescigno, and all the speakers who had accepted and kept their commitment.
This Journal reports, as usual, the abstracts of the oral presentations of the speakers and those of the poster presenters.
Moreover, we'll be able to report in 3 weeks the highlights of the symposium, in collaboration with the CIBIO Department of the University of Trento and, for the first time, the European School of Oncology (ESO). These highlights will be presented on June 12th, in an ESO event to a potentially even larger audience.
Finally, I can't thank enough all those who made a key contribution to the realization of this event, in particular the staff of the Pezcoller Foundation, the organizing agency Orikata and Jam Session technical services.
I wish everyone a good work, hoping to meet again, in presence, next year.

Enzo Galligioni

32nd virtual Pezcoller Symposium

AGING AND CANCER

June 21-22, 2021

Co-Organizers:
Alberto Bardelli, Fabrizio D'Adda di Fagagna, Giannino Del Sal, David Livingston, Massimo Loda, Stefano Piccolo, Maria Rescigno

* Central European Time (CEST)
** Virtual poster presentations can be viewed upon accessing the reserved area, during the entire duration of the Symposium

MONDAY JUNE 21, 2021

13:30 Enzo Galligioni **Welcome**
13:40 David Livingston **Focus & Goals**

The Enrico Mihich Lecture

Chair: **David Livingston**

13:50 Hans Clevers *Human organoids as models for disease*
14:35 Discussion

Session 1: Connections Between Aging and Cancer

Chair: **Maria Rescigno**

14:50 Marc Gunter *Obesity, metabolic health and cancer: molecular epidemiologic studies*
15:15 Discussion

15:30 *Ten-minute break*

15:40 Judith Campisi *Aging and cancer: Rival demons or reluctant allies?*
16:05 Discussion

16:20 Fiona Watt *Understanding cell heterogeneity in multi-layered epithelia*
16:45 Discussion

17:00 Juan Carlos Belmonte *Aging and Regeneration*
17:25 Discussion

17:40 *Ten-minute break*

Session 2: Longevity, Senescence, Premature Aging and Cancer Genomics

Chair: **Giannino Del Sal**

17:50 Anne Brunet *Understanding and modeling aging*
18:15 Discussion

18:30 Manuel Serrano *Understanding and controlling in vivo reprogramming for rejuvenation*
18:55 Discussion

19:10 Tom Misteli *Learning from premature aging*
19:35 Discussion

19:50 Serena Nik Zainal *Somatic mutagenesis in normal and cancer cells*
20:15 Discussion

20:30 *Adjourn*

TUESDAY JUNE 22, 2021

Session 3: Thematic Insight Lecture: Aging as a Cancer Risk Factor

Chair: Stefano Piccolo

13:30 Gerard Evan — *Aging as a Cancer Risk Factor*
14:15 Discussion

Session 4: Neoplastic Outcomes Associated with Aging

Chair: **Massimo Loda**

14:30 Myles Brown — *Essential cancer genes and cistromes*
14:55 Discussion

15:10 Peter Miller — *Clonal hematopoiesis in malignant and non-malignant disease*
15:35 Discussion

15:50 *Ten-minute break*

Chair: **Fabrizio D'Adda di Fagagna**

16:00 Phil Jones — *Pro- and anti-oncogenic mutants colonise normal epithelia*
16:25 Discussion

16:40 Jan Karlseder — *How proliferative boundaries prevent cancer initiation*
17:05 Discussion

17:20 Joachim Lingner — *Telomere maintenance and the making of TERRA R-loops at chromosome ends*
17:45 Discussion

18:00 *Ten-minute break*

18:10 Luca Magnani — *Tumour dormancy and ageing in hormone dependent breast cancer*
18:35 Discussion

Session 5: Unexplained Outbreaks of Colorectal Cancer in Young Adults

Chair: **Alberto Bardelli**

18:50 Gianluca Mauri — *The emerging challenge of early-onset colorectal cancer*
19:15 Discussion

19:30 Massimo Loda — *3 best Poster Presentation and Discussion*

20.00 David Livingston — *Concluding Remarks*

INVITED SPEAKERS

FACULTY

- **Bardelli Alberto**
  Candiolo Cancer Institute, University of Torino, IT
- **Belmonte Juan Carlos Izpisua**
  Salk Institute for Biological Studies, La Jolla, CA
- **Brunet Anna**
  WU Tsa Neuroscience Institute Stanford University, CA
- **Brown Myles**
  Center for Functional Cancer Epigenetics, Dana Farber Cancer Institute, Boston, MA
- **Campisi Judith**
  Buck Institute for Research on Aging, Novato, and Lawrence Berkeley National Laboratory, Berkeley, CA
- **Clevers Hans**
  Hubrecht Institute, University of Utrecht, NL
- **D'Adda di Fagagna Fabrizio**
  IFOM – the FIRC Institute of Molecular Oncology, Milano, IT
- **Del Sal Giannino**
  ICGEB, University of Trieste, IT
- **Evan Gerard**
  University of Cambridge, Cancer Center, UK
- **Gunter Marc**
  Imperial College London, UK
- **Karlseder Jan**
  Salk Institute for Biological Studies, La Jolla, CA
- **Jones Phil**
  University of Cambridge, Wellcome Trust Sanger Institute, UK
- **Lingner Joachim**
  Ecole Polytechnique Fédérale de Lausanne, Lausanne, CH
- **Livingston David**
  Dana Farber Cancer Institute, Boston, MA
- **Loda Massimo**
  Weill Cornell Medicine, New York, NY
- **Magnani Luca**
  Imperial College Hammersmith, London, UK
- **Mauri Gianluca**
  Università degli Studi di Milano, Grande Ospedale Metropolitano Niguarda, Niguarda Cancer Center, Milano, IT
- **Miller Peter**
  Dana Farber Cancer Institute, Boston, MA
- **Misteli Thomas**
  National Institute of Health, National Cancer Institute, Bethesda, MD
- **Nik-Zainal Serena**
  University of Cambridge MRC Cancer Unit, UK
- **Piccolo Stefano**
  Department of Molecular Medicine, University of Padova, IT
- **Rescigno Maria**
  Humanitas University, Milano, IT
- **Serrano Manuel**
  Institute for Research in Biomedicine, Barcelona, SP
- **Watt Fiona**
  Centre for Stem Cells and Regenerative Medicine, King's College London, UK

32nd virtual Pezcoller Symposium

Aging and Cancer

June 21-22, 2021

ABSTRACTS OF ORAL PRESENTATIONS

Modeling development and disease with human organoids

Hans Clevers

Hubrecht Institute, Utrecht, the Netherlands

Techniques for culturing functional human breast epithelium in three-dimensional (3D) matrices have been championed for more than 30 years by Mina Bissell. Additionally, around a decade ago, Sasai and colleagues pioneered pluripotent stem cell (PSC)-based technology to create organoids that mirror specific parts of the central nervous system (CNS). Lancaster and Knoblich modified this technology and provided particularly notable examples of "mini-brain" structures. Although PSCs can be used to model everything ranging from tissues to organismal development, adult stem cells (ASCs) can also be isolated to generate organoid models of the primary tissues in which they reside. Specific growth factor cocktails allow long-term expansion of ASC organoids by mimicking the organ stem cell niche, as first established for mouse and human intestine and airway epithelium. The organoid structures generated from PSCs and ASCs reflect crucial tissue features in terms of overall architecture, the collection of differentiated cell types, and tissue-specific function. Organoids thus represent a model system that can be compared to traditional genetically engineered mouse models (GEMMs), cell lines, and patient-derived xenografts (PDXs).
As a definition, organoids are microscopic self-organizing, three-dimensional structures that are grown from stem cells *in vitro*. They recapitulate many structural and functional aspects of their *in vivo* counterpart organs. This versatile technology has led to the development of many novel human cancer models. It is now possible to create indefinitely expanding organoids starting from tumor tissue of individuals suffering from a range of carcinomas. Alternatively, CRISPR-based gene modification allows the engineering of organoid models of cancer through the introduction of any combination of cancer gene alterations to normal organoids. When combined with immune cells and fibroblasts, tumor organoids become models for the cancer microenvironment enabling immune-oncology applications. Emerging evidence indicates that organoids can be used to accurately predict drug responses in a personalized treatment setting. I will review the current state and future prospects of the rapidly evolving tumor organoid field.

Obesity, metabolic health and cancer: molecular epidemiologic studies

Marc Gunter

Imperial College London, IARC

Adiposity and Type 2 diabetes are established risk factors for a growing number of malignancies including cancers of the colorectum, gallbladder, pancreas, endometrium, postmenopausal breast, thyroid, hepatocellular and renal cell carcinoma and oesophageal adenocarcinoma. Obesity is associated with significant metabolic and endocrine abnormalities including alterations in sex hormone metabolism, insulin signalling, and adipokines/inflammatory pathways. All three mechanisms influence the balance between cell proliferation and apoptosis and have been linked to cancer development in both experimental and observational studies. However, it is likely that other, hitherto unrecognised molecular pathways mediate the adiposity-cancer association. In this presentation I will discuss new molecular epidemiologic

approaches to understanding the link between obesity, metabolic dysfunction and cancer, highlighting our ongoing work that exploits metabolomics, genomic and proteomic tools within the framework of prospective cohort studies and randomized controlled trials.

Aging and cancer: Rival demons or reluctant allies?

Judith Campisi

Buck Institute for Research on Aging, Novato CA USA and Lawrence Berkeley National Laboratory, Berkeley CA USA

Age is the largest risk factor for developing a host of pathologies, ranging from neurodegeneration to cancer. Because age-related diseases are so diverse, it is likely that one or a few basic aging processes drive most, if not all, aging phenotypes and pathologies. One candidate for such a process is cellular senescence, a complex cell state adopted by cells in response to both physiological and stressful stimuli. Senescent cells arrest proliferation (growth), essentially irreversibly, and develop a multi-faceted senescence-associated secretory phenotype (SASP).
The senescence response is likely an example of antagonistic pleiotropy -- an evolutionary tradeoff that provides benefit to young organisms, but is deleterious to old organisms. Among the benefits, the senescence growth arrest is a powerful tumor suppressive mechanism, protecting young organisms from developing cancer. In addition, the SASP helps fine-tune certain processes during embryonic morphogenesis, the initiation of parturition and the repair of injured tissues. Among the deleterious effects, the growth arrest can also deplete tissues of functional stem cells and, importantly, the SASP can fuel both local and systemic inflammation. Age-related inflammation, also termed inflammaging, is a major risk factor for developing age-related diseases, including, ironically, late-life cancer.
Transgenic mouse models that allow the selective elimination of senescent cells, cultures of human cells and organoids, and, more recently, the development of new drugs termed senolytics, show that senescent cells do indeed drive many of the pathologies associated with aging. With regard to cancer, the SASP includes factors that not only promote cancer cell proliferation, but also cancer cell invasion and metastasis. In addition, many genotoxic and cytotoxic anti-cancer chemotherapies, which successfully kill many cancer cells, can also induce a senescence response in both tumor cells and normal cells. These therapy-induced senescent cells can additionally serve to fuel the development of malignant phenotypes in residual living tumor cells.As the development of senolytic drugs proceeds, the use of these drugs as adjuvants to the commonly used anti-cancer drugs, may help prevent cancer recurrence.

Understanding cell heterogeneity in multi-layered epithelia

Fiona M. Watt

Centre for Stem Cells and Regenerative Medicine, King's College London

Advances in single cell technologies such as RNA-sequencing provide new opportunities to understand the cellular heterogeneity of different tissues. The Human Cell Atlas is a global initiative to create a comprehensive reference map of all human cells in the body as a basis for understanding human physiology in health and disease. As part of this initiative my lab and others are collaborating to study human skin from healthy donors and patients with psoriasis or atopic dermatitis. Our analysis is providing new insights into the nature of the epidermal stem cell compartment. Complemented by mechanistic studies of how cells transition between different states, we are starting to gain new understanding of this fascinating tissue. Specifically, the existence of different subpopulations of stem cells, which was predicted from clonal analysis of cultured human epidermal cells, is confirmed by Human Cell Atlas data. In addition, the nature of the transition from stem to differentiated cell and the role of protein phosphatases in that transition are consistent with single cell RNA sequencing datasets.

Aging and Regeneration

Juan Carlos Izpisua Belmonte

Salk Institute for Biological Studies, La Jolla, CA

Ageing is characterized by the functional decline of tissues and organs and the increased risk of ageing- associated disorders. Several 'rejuvenating' interventions have been proposed to delay ageing and the onset of age- associated decline and disease to extend healthspan and lifespan. These interventions include metabolic manipulation, partial reprogramming, heter-

ochronic parabiosis, pharmaceutical administration and senescent cell ablation. As the ageing process is associated with altered epigenetic mechanisms of gene regulation, such as DNA methylation, histone modification and chromatin remodelling, and non- coding RNAs, the manipulation of these mechanisms is central to the effectiveness of age- delaying interventions. I will discuss some of epigenetic changes that occur during ageing and the rapidly increasing knowledge of how these epigenetic mechanisms have an effect on healthspan and lifespan extension, and will outline questions to guide future research on interventions to rejuvenate the epigenome and delay ageing processes.

Understanding and modeling aging

Anne Brunet

WU Tsa Neuroscience Institute Stanford University

Aging is associated with a decline in tissue function and the onset of a constellation of diseases. We are interested in understanding aging, with a particular focus on brain aging. Because aging is complex, we use organisms with diverse lifespans – the worm C. *elegans*, the African killifish, the mouse, and cells from mice and humans. We are interested in identifying epigenetic and metabolic pathways involved in delaying aging in response to external stimuli, including nutrients and the opposite sex. Our lab is also interested in using mouse models to address complex questions about mammalian aging, notably the regulation of regenerative neural stem cells and their progeny during aging. Finally, we are pioneering the naturally short-lived African killifish as a new model to identify principles underlying vertebrate aging and "suspended animation". We hope that these discoveries will identify new strategies to delay, suspend, or even reverse aspects of aging and age-related diseases.

Understanding and controlling in vivo reprogramming for rejuvenation

Manuel Serrano

Institute for Research in Biomedicine (IRB), Barcelona, Spain

My laboratory has pioneered the use of the Yamanaka factors for reprogramming *in vivo*. This consists on directly reprogramming cells in their natural context within the organism, thus bypassing the need of *in vitro* treatments or cell transplantation. We are using our "reprogrammable" mice to learn how to control *in vivo* reprogramming. We have previously reported specific pharmacological interventions that improve, and others that impair, *in vivo* reprogramming. I will present a novel intervention that greatly improves reprogramming by simply supplementing the diet with a particular vitamin. I will also present data on the rejuvenating potential of a single cycle of transient reprogramming in naturally aged mice.

Learning from premature aging

Tom Misteli

National Cancer Institute, NIH, Bethesda, MD

Aging is a fundamental biological process and a major risk factor for many of the most common human diseases including cardiovascular disease and cancer. While animal model systems have been widely used to characterize the molecular basis of aging, the study of human aging is complicated by the inability to perform targeted *in vivo* experiments. Naturally occurring premature aging diseases are a powerful tool to probe human aging mechanisms. To this end, we are investigating the ultra-rare Hutchinson-Gilford Progeria Syndrome (HGPS), which is caused by mutation in *LMNA*, encoding the major nuclear architectural protein, lamin A. I will discuss molecular and cellular mechanisms of HGPS and its potential relevance to human aging and cancer.

Somatic mutagenesis in normal and cancer cells

Serena Nik Zainal

MRC Cancer Unit, University of Cambridge, UK

Mutational signatures are the imprints of DNA damage and DNA repair processes that have been operative during tumorigenesis. They are biologically informative, reporting on the processes that have contributed to the developmental history of each patient's cancer. In this lecture, on behalf of my team and my collaborators, I shall provide an update on the field, focusing on validation of these abstract mathematical concepts, untangling the mechanisms underpinning mutation patterns in human somatic cells, and

describing the new insights that we have gained through combinations of computational analysis and experiments in cell-based systems. We showcase a recent assessment of the extent of mutagenesis in a human stem cell model system and discuss the implications for the research community.

Cancer, Aging, Damage and Repair

Gerard I. Evan

Department of Biochemistry, University of Cambridge, UK

Cancers are genetically complex, rogue somatic clones that arise by aleatory accumulation of mutations that disrupt the intra- and extracellular signalling networks that govern somatic cell growth, proliferation, survival and invasion. Because of their pivotal importance in instructing tissue ontogeny, maintenance and repair, these signalling networks have evolved to be highly robust and functionally redundant. Unfortunately, such remarkable plasticity and capacity for self-organization and self-correction comes at a cost: redundant networks offer an abundance of targets for oncogenic mutation - evident in the bewildering inter- and intra-tumoural heterogeneity of human cancers - and their innate robustness contributes to the high rate of relapse in patients treated with targeted anti-cancer drugs. Such bewildering inter- and intra-tumoural heterogeneity of human cancers has occasioned huge efforts to catalogue and categorize human tumours and, on the basis of their idiosyncrasies, develop tailored precision therapies. However, this preoccupation with the differences between, and within, cancers overlooks the equally remarkable, and oft-overlooked, fact that cancers of a particular tissue type share great phenotypic similarity with each other, while typically appearing quite different from cancers arising in other tissues, even when they share the same oncogenic mechanisms. We are interested in where and how these tissue-specific tumour phenotypes are encoded, why they arise and why they look the way they do, and what they tells us about the essential underlying *commonality* shared by human cancers. The answers to these questions not only point to the possibility of more generalized and effective cancer therapies for the future but also offer an answer to why targeted therapies work in the first place – in particular, why tumour cells appear preternaturally "addicted" to their oncogenic mutations.

Essential cancer genes and cistromes

Myles Brown

Center for Functional Cancer Epigenetics, Dana-Farber Cancer Institute, Boston, MA

Steroid hormones mediate critical lineage-specific developmental and physiologic responses. While estrogen is required for normal breast development, the genes regulated by estrogen and the genomic targets of the estrogen receptor (ER) are altered in ER+ breast cancers. The requirement of most breast cancers for estrogen has led to the development of endocrine therapies that block ER action. While initial endocrine interventions are successful, in the advanced disease setting resistance to ER-targeted therapy almost invariably arises. As with other targeted therapies, gain of function mutations play a significant role in the development of therapeutic resistance to ER directed treatments in breast cancer. In addition, pathways downstream of ER are activated in cases in which ER itself is not genetically altered suggesting that these pathways remain essential. Activation of these pathways may depend on mutations in other transcription factors involved in steroid receptor networks or chromatin modifying enzymes involved in transcriptional regulation mediated by ER. These mutations may be present in the protein coding regions of the genes or in the cis-regulatory elements regulating expression of key genes in the pathways. Renewed success in targeting ER and promising advances in inhibiting the activity of the chromatin modifying enzymes provides new opportunities for the treatment of patients with breast cancer. Using genome-wide CRISPR-Cas9 knockout screens we have identified the genes essential for hormone-dependent breast cancer growth including key genes in the receptor-centered transcriptional networks.

Although large sets transcription factor binding sites or cistromes have been identified across the human genome, defining which of these sites is functional in a given condition remains challenging. Using CRISPR-Cas9 knockout screens and gene essentiality or fitness as the readout, we systematically investigated the essentiality of more than 10,000 FOXA1 and CTCF binding sites in cancer cells. We found that essential FOXA1 binding sites act as enhancers to orchestrate the expression of nearby essential genes through the binding of lineage-specific transcription factors. In contrast, CRISPR screens of the CTCF cistrome revealed two classes of essential binding sites. The first class of essential CTCF binding sites act like FOXA1 sites as enhancers to regulate

the expression of nearby essential genes, while a second class of essential CTCF binding sites was identified at TAD boundaries and display distinct characteristics. Using regression methods trained on the screening data and public epigenetic profiles, we developed a model to predict essential cis-elements with high accuracy. The model for FOXA1 essentiality correctly predicts non-coding variants associated with cancer risk and progression. Taken together, CRISPR screens of cis-regulatory elements can define the essential cistrome of a given transcription factor and can inform the development of predictive models of cistrome function. In addition, defining the essential cistrome may shed light on the noncoding regions of the genome most likely to harbor oncogenic driver mutations.

Clonal hematopoiesis in malignant and non-malignant disease

Peter Miller

Dana Farber Cancer Institute, Boston MA

Over the lifetime of an organism, cell division results in the acquisition of mutations, some of which confer a fitness advantage to the cells in which they arise. Clonal hematopoiesis describes the expansion of clonally-derived hematopoietic cells in the peripheral blood. Clonal hematopoiesis of indeterminate potential (CHIP), defined by the presence of leukemia-associated mutations with a variant allele fraction (VAF) of at least 2%, is associated with an increased risk of hematologic malignancy and elevated mortality among healthy individuals and patients with cancer.[1-3] In an aging population, the most commonly mutated genes in CHIP include *DNMT3A*, *TET2*, and *ASXL1*. However, CHIP is significantly more common in individuals who have received cytotoxic therapy, and the clonal cells are more likely to carry mutations in genes that regulate the DNA damage response including *PPM1D* and *TP53*.[4,5]

PPM1D is a gene on chromosome 17q that encodes for a PP2C family serine/threonine phosphatase. PPM1D has been most extensively studied as a negative regulator of the DNA damage response (DDR) and P53 activation via dephosphorylation of pathway members including P53, MDM2, CHK1, CHK2, ATM, ATR, and γH2AX.[6] PPM1D was subsequently shown to negatively regulate other pathways including P38 MAPK, NF-κB, and mTOR, suggesting its ability to influence numerous cellular processes.[7-9] Consistent with this biology, *PPM1D* is both amplified and recurrently mutated in numerous oncologic contexts.

PPM1D mutations found in clonal hematopoiesis and therapy-related myeloid malignancies are largely truncating mutations in the c-terminus of the protein. We have previously shown that these mutations result in a truncated protein less amenable to proteasomal degradation, leading to increased activity. In turn, these mutations allow for enhanced suppression of the DNA damage response and resistance to cytotoxic therapy.[10]

Using genetically engineered mouse models of truncating *PPM1D* mutations and *PPM1D* knockout, we have now shown that these activating mutations confer a selective advantage during the stress of transplantation and in the presence of cytotoxic therapies. Furthermore, genetic loss of *PPM1D* or pharmacologic inhibition of PPM1D sensitizes myeloid leukemias to a variety of chemotherapeutic agents. These data, in combination with the known genetic activation of *PPM1D* in numerous cancers, suggest that PPM1D may be a meaningful therapeutic target. To augment drug discovery and medicinal chemistry efforts, which have been limited by the lack of a crystal structure and thus understanding of the structural features of PPM1D, we have performed computational and functional genetic experiments on *PPM1D* to characterize the domains and residues required for protein function. Using biophysical and biochemical assays, including hydrogen-deuterium exchange mass spectrometry and analytical ultracentrifugation, we have further refined our understanding of the enzymatic properties of PPPM1D and defined its conformational states. Finally, we have determined the mechanism by which an allosteric binder of PPM1D inhibits its catalytic function.

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Pro- and anti-oncogenic mutants colonize normal epithelia

Phil Jones

Wellcome Sanger Institute and University of Cambridge, UK

Human epithelial tissues accumulate a high burden of cancer associated mutations with age (1-3). The positive genetic selection of oncogenic mutations argues that they increase the competitive fitness of proliferating cells to generate expanding clones. Hence, aging normal tissues such as the oesophagus become a patchwork of mutant clones competing for space and survival, with the fittest clones expanding by eliminating their less-competitive neighbours. Clones expand until they encounter cells of similar fitness, at which point their behaviour reverts towards that of wild type cells (4). Some mutant genes such as *TP53* almost universal in cancer but comparatively rare in normal tissue, but others like *NOTCH1* colonize the majority of normal oesophagus by middle age but are rare in cancer (2). Mouse models show biallelic disruption of *p53* results in chromosomally unstable clones in normal epithelium and promotes tumour growth, while *Notch1* loss slows tumour expansion, arguing this mutation protects against transformation.

Little is known about how the dynamic competition of mutants in normal epithelia influences the earliest stages of tumorigenesis. Using whole organ single cell resolution 3D imaging we followed the fate of cohorts of nascent, microscopic, pre-malignant tumours in a mouse model of oesophageal carcinogenesis, finding most are rapidly lost. Deep-sequencing of early and later tumours showed evidence of genetic selection on the surviving neoplasms, which developed features of dysplasia. There was no evidence of tumour cell death, decreased proliferation, or an anti-tumour immune response but induction of highly competitive clones in transgenic mice increased early tumour removal, while pharmacologically inhibiting clonal competition reduced tumour loss. These results support a model where survival of early neoplasms depends on their competitive fitness relative to that of mutant clones in the surrounding normal tissue. Mutant clones in normal epithelium have an unexpected anti-tumorigenic role in purging early neoplasms through cell competition, thereby preserving tissue integrity. This may contribute to comparative rarity of cancers compared with the level of oncogenic mutations in aging normal tissues.

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How Proliferative Boundaries Prevent Cancer Initiation

Joe Nassour, Tobias Schmidt, Reuben Shaw and Jan Karlseder

Salk Institute for Biological Studies, La Jolla, CA

With each cell division telomeres, the ends of eukaryotic chromosomes, shorten due to the "end replication problem". Primary human cells with shortened telomeres stop dividing and enter replicative senescence to prevent genomic instability. Inactivation of the p53 and retinoblastoma protein (Rb) pathways allows cells with shortened telomeres to bypass replicative senescence and divide further, until telomeres are extremely short and cells enter a second plateau called replicative crisis. During crisis the majority of cells succumb to cell death. However, very rarely (1 in 10 to 30 million human fibroblast crisis cells), individual clones in the crisis population escape from crisis through unknown mechanisms or by the activation of a telomere maintenance mechanism. These immortal, post-crisis clones display unlimited replication capacity, one essential hallmark in the progression from a primary to a cancer cell. Thus, both replicative senescence and replicative crisis are two major barriers preventing the transformation of primary to cancer cells.

Recently we made the discovery that cells in replicative crisis die not as generally anticipated by apoptosis, but by autophagic activation. We could demonstrate that telomeric damage activates the autophagy machinery, as opposed to intrachromosomal damage, which signals towards apoptosis. Moreover, depletion of the autophagy proteins ATG5 or ATG7 or the cytosolic DNA sensor cGAS/STING machinery, allowed cells to bypass the crisis plateau. As consequence of crisis bypass genome instability accumulated, pointing at autophagy as potent tumor suppres-

sor during the earliest stages of cancer development and allowing the speculation that cells have to attenuate autophagy at least temporarily during oncogenic transformation. Currently we are exploring the exact signaling pathways between telomere shortening and autophagy activation, focusing on the cytoplasmic sensors that recognize nucleic acids in the cytoplasm. Furthermore, we are investigating how the inflammation response stimulated by dysfunctional telomeres leads to autophagy activation and cell death, thereby preventing cancer initiation.

Telomere maintenance and the makings of TERRA R-loops at chromosome ends

Marianna Feretzaki, Rita Valador Fernandes, Galina Glousker, Suna In, Eftychia Kyriacou, Chih-Yi Lin, Thomas Lunardi, Samah Matmati, Anna Näger, Trang Nguyen, Joachim Lingner

EPFL, Lausanne, Switzerland.

Telomeres correspond to the physical ends of eukaryotic chromosomes. They protect chromosome ends from DNA degradation and rearrangements. Telomeres also serve as cellular clocks. Due to the end replication problem, they shorten with every round of DNA replication limiting the lifespan of most human somatic cells. Telomere shortening is overcome in stem cells and in cancer by the telomerase enzyme. In addition, telomere shortening can be counteracted by homologous recombination (HR) engaging telomeric DNA repeats of different chromosome ends. The latter is particularly relevant in so-called ALT cancer cells which counteract telomere shortening in the absence of telomerase.
Telomeres are associated with a large number of proteins which mediate telomere functions. The telomeric long noncoding RNA TERRA, which is transcribed at chromosome ends is another important telomeric component. Its roles have just started to be unraveled. Among others, TERRA has been implicated in modulating chromatin structure and DNA damage signaling when telomeres are damaged. TERRA also stimulates telomere elongation by homology directed repair in ALT cancer cells. Our recent data demonstrate that TERRA associates with telomeres forming DNA:RNA hybrid structures known as R-loops. Significantly, these R-loops are generated post transcription requiring the RAD51 DNA recombinase. Therefore, the recruitment of TERRA seems to occur through a process that strongly resembles the strand-invasion and homology-search mechanism exploited in all living organisms during DNA repair by HR. TERRA preferentially associates with short telomeres where TERRA R-loops may stimulate their preferential elongation by recombination. On the other hand, TERRA R-loops can also interfere with telomere maintenance when present in S phase as they clash with the semiconservative DNA replication machinery. To prevent this, several telomeric proteins counteract TERRA R-loops. In this talk, I will report on factors that regulate TERRA association and R-loop formation at chromosome ends and how this impinges on telomere maintenance by DNA replication and homology directed repair.

Tumor dormancy and aging in hormone dependent breast cancer

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Hormone dependent Breast cancers are the most commonly diagnosed malignancies in women. Long-term survival of these patients has remarkably improved thanks to adjuvant endocrine therapies (ET), but HDCs now outweigh all other cancer types in terms of late tumor relapses (> 5 years). Our view is that ETs and bone microenvironment induce epigenetic dormancy in most disseminated cancer cells. Endocrine therapies are designed to starve tumour cells by lowering circulating hormones (aromatase inhibitors) or by blocking hormone-dependent transcription (Tamoxifen and Fulvestrant). My lab is interested in exploring the connection between therapeutic intervention, epigenetic decay and dormancy. We are fascinated by the fact that the hormonal profile of an ageing woman might have strong parallel to systemic endocrine treatments. We use single cell transcriptomics, epigenetic and lineage tracing to investigate the events underlying non genetic adaptation to systemic therapies. The underlining question we will discuss in this lecture is: is dormancy a developmental pathway that cancer cells borrow in response to treatment? Does dormancy stability play a role in cancer initiation? How can we translate this knowledge in better patient outcome?

The emerging challenge of early-onset colorectal cancer

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Colorectal cancer (CRC) is the third most common cancer and cause of cancer death worldwide in both men and women. In the last decades, the overall CRC incidence and mortality in the USA and Europe has declined. Conversely, since 1994 CRC incidence in individuals younger than 50 years has been increasing by around 2% per year. This trend has been described worldwide and it is not confined only to high-income areas of the world. The reasons underlying the increase of early-onset CRC (EO-CRC) are still unknown.

EO-CRC are usually characterized by more aggressive clinical and pathology features and often initially diagnosed at later stages of disease. Even if there is a well-known higher prevalence of hereditary and familiar CRC among the younger population, the majority of EO-CRCs are sporadic. This is true even when considering only patients younger than 40 years of age.

Many studies recently assessed the molecular landscape of EO-CRC and did recognize some peculiarities such as a lower prevalence of BRAFV600E mutations and higher prevalence of CMS1 molecular signature.

Data on EO-CRC outcome and prognosis are still very incomplete and not conclusive. However, it appears that the younger age the worse prognosis is. Perspective trials exploiting molecular and immunological treatments are warranted to clarify this aspect.

In this presentation we summarize the main epidemiological, pathological, and clinical aspects of EO-CRC, and envisage future research perspectives based on initial data from an ongoing observational study at Grande Ospedale Metropolitano Niguarda and Università degli Studi di Milano and Università degli Studi di Torino aimed at investigating molecular, genomic, and immunological features of EO-CRC.

ABSTRACTS OF POSTERS

Annotating and overcoming resistance to NAMPT inhibitors in cancer cells

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Nicotinamide phosphoribosyltransferase (NAMPT) is a key metabolic enzyme in NAD+ synthesis found upregulated in several tumors. Thus, NAMPT inhibitors as FK866, offers an appealing approach for anticancer therapy, even though drug resistance remains an unsolved problem in cancer chemotherapy.
To study the process of acquired resistance to the metabolic drug FK866, the triple negative breast cancer cell line MDA-MB-231 was exposed to increased concentrations of the drug, until becoming resistant (MDA-MB-231-R) to FK866. In this model, NAMPT is not mutated and MDA-MB-231-R are not sensitive to verapamil or cyclosporin A co-treatment with FK866, excluding a potential role of increased efflux pumps activity as a mechanism of resistance. Similarly, the silencing of the enzyme Nicotinamide Riboside Kinase 1 (NMRK1; phosphorylating Nicotinamide Riboside to Nicotinamide Mononucleotide) in MDA- MB-231-R does not increase FK866 toxicity excluding compensatory mechanism of NAD production.
Mitochondrial substrate utilization revealed a higher rate of pyruvate and malic acid metabolism in MDA- MB-231-R, but Pyruvate dehydrogenase E1 subunit (PDH1) and the mitochondrial pyruvate carrier (MPC) were not overexpressed in the resistant model. Co-treatment of MDA-MB-231-P with the MPC inhibitor UK5099 and FK866 induces a FK866 resistant phenotype possibly indicating a shift towards a more glycolytic metabolism.
Taken together, these results unravel MPC as a potential mediator of FK866 resistance in the acute phase of the treatment, that is later sustained by mitochondrial adaptation and increased spare respiratory capacity.

Conditioned medium from Doxorubicin-induced senescent Wi-38 fibroblasts increases Osteosarcoma cell growth and migration: pretreatment with Quercetin, reducing cell senescence, decreases these effects

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Background
Cellular senescence is tumour-suppressive mechanism that prevents the proliferation of premalignant cells. Several studies show that senescent cells (SCs) can promote tumour progression and other age- related diseases via the senescence-associated secretory phenotype (SASP). Potential strategies for mitigating the effects of SCs include removing and interfering with their adverse effects by targeting the SASP. In this framework, we have studied the ability of Quercetin, a natural bioactive flavonoid, in reducing the onset of Doxorubicin-induced senescence in Wi-38 fibroblasts. Further, we have investigated the capacity of conditioned media (CM) from Doxo-induced senescent fibroblasts (DSF) and Quercetin-pretreated DSF (QDSF) to induce growth and migration of osteosarcoma cells (OS). Methods
WI-38 cells were incubated with/without Quercetin 40 μM for 24h and then treated with Doxo 50nM for 48h. 72h after Doxo-treatment, the senescent phenotype was evaluated by analyzing the expression of senescence-associated ß-galactosidase activity (SA-ß-gal), cell cycle arrest markers, and senescence-associated heterochromatin foci (SAHF). Moreover, CM from DSF (DSF-CM) and QDSF (QDSF-CM) were used to treat OS (U2OS cell line). CM coming from no treated fibroblasts was used as control.
Results
QDSF shows a significant reduction of SA-ß-gal activity, cell cycle arrest markers, and SAHF presence compared to DSF. Quercetin increases

the cells' antioxidant capacity and reduces ROS production, partially protecting the cells from Doxo-induced damage and senescence. Further, we have observed that U2OS cells treated with DSF-CM significantly increase their growth and migration, while those treated with QDSF-CM behaved like the control cells.

Conclusion

The effects of SASP from therapy-induced senescent fibroblasts on tumor microenvironment remains yet to be clarified. However, our study shows that DSF-CM significantly increased U2OS proliferation and migration, promoting aggressive behaviour, while QDSF-CM does not induce the same aggressiveness. Quercetin effectiveness in preventing/reducing Doxo-induced senescence can be due to its antioxidant capacity, but further investigation is necessary. Our results might pave the way for the use of Quercetin to contrast the adverse effects of therapy-induced senescence on OS microenvironment and might be an excellent strategy to reduce therapy toxicity and metastatic spread, two of the significant causes of treatment failure.

Regulation and function of tumor suppressor DAB2IP in the cellular response to mechanical inputs

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The crosstalk between tumor cells and their microenvironment is a major determinant of cancer progression. Thus, understanding and eventually interfering with the dynamic interplay between external cues and cellular responses may lead to more efficient chemotherapeutic approaches.

The tumor suppressor DAB2IP encodes a RasGAP and cytoplasmic adaptor that controls specificity, amplitude, and duration of intracellular signaling events in response to multiple extracellular inputs such as inflammatory cytokines, growth factors and hormones. Accordingly, loss of DAB2IP function amplifies multiple oncogenic pathways and fosters cancer aggressiveness.

In addition to secreted factors, also mechanical forces from the extracellular environment significantly affect tumorigenesis, metastasis and chemoresistance; here we asked if DAB2IP may contribute to sensing and/or reacting to mechanical inputs, and if loss of function of DAB2IP would alter the response of cancer cells to mechanical cues.

Using tumoral and normal mammary cell lines, we found that mechanical inputs such as substrate (ECM) stiffness and cell-cell contact can dynamically modulate DAB2IP. We found that DAB2IP protein levels are reduced when cells are grown on a soft substrate, suggesting that ECM rigidity positively affects its expression. We also observed that cell adhesion to the substrate sustains DAB2IP expression – possibly via Focal Adhesions (FAs). Similarly, integrity and contractility of the actin cytoskeleton is required to maintain DAB2IP levels. Finally, we found that DAB2IP protein is noticeably reduced in low density cultures, while upregulated when cells are grown to high concentration, indicating that also cell-cell contact controls DAB2IP levels - likely via Adherens Junctions (AJs).

Inspired by this evidence, we asked whether DAB2IP may in turn modulate the cell's mechanical properties and/or the cell's response to mechanical inputs. We found that DAB2IP knockdown cells, when grown to confluency, have larger nuclei and a higher Young's modulus (rigidity). Under the same conditions, DAB2IP knockdown cells display enhanced activation of YAP, a key transcriptional effector of mechanical inputs and powerful oncogene. These observations suggest that DAB2IP might contribute to restrain YAP activity under conditions of contact-inhibition. This intriguing hypothesis, and its possible mechanism, are currently under investigation.

Role of Base Excision Repair proteins in cancer cell senescence through control of SASP factors gene expression and miR130b regulation

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Cellular senescence is an irreversible cell cycle arrest caused by different stresses, such as DNA damage, oxidative stress, oncogene expression, which are potentially deleterious for the cells. In response to these stimuli, the p53-p21 and p16-Rb pathways are activated, promoting cell cycle arrest. The 'senescence-associated secretory phenotype' (SASP) is one of the most important molecular features of senescence, in which IL-6 and IL-8 mainly participate in regulating tissue microenvironment. Cellular senescence is associated with tumor progression and chemoresistance. Ineffective DNA repair

compromises ageing cell clearance generating a chronic pro-inflammatory condition. It has been demonstrated that the Base Excision Repair (BER) pathway, in particular the Apurinic/apyrimidinic endodeoxyribonuclease 1 (APE1) protein, is involved in the induction of the intrinsic cell senescence. Currently, an involvement of BER in extrinsic cancer cell senescence is still unknown. Recently, the use of senolytic compounds has been employed as a novel pharmacological strategy to selectively eliminate senescent cells. In the present work, we explored a possible implication of BER enzymes in extrinsic cell senescence of A549 cancer cells. For this purpose, we first set-up an extrinsic cancer cell senescence model, by using cisplatin (CDDP) as genotoxicant. We found that APE1 was down-regulated, in a proteosomal-dependent manner, during cancer cell senescence onset, while no significant variations were obtained for the other BER enzymes. Moreover, APE1 contributed to the onset of senescence, by inducing SASP factors (IL-6 and IL-8) in the early phase of the process. Of interest APE1 controlled the expression of miRNAs involved in cell senescence. We observed that APE1-regulated miR, i.e. miR-130b which regulates p21 expression, is down-regulated in line with APE1 expression, leading to an upregulation of its target p21 and a promotion of the senescence process. Interestingly, treatment of senescent cells with APE1 inhibitors sensitizes cancer cells to CDDP-treatment. These data indicate that APE1 participates in the early phases of senescence through the induction of SASP factors and microRNA dis-regulation. Moreover, we demonstrated the senolytic activity of APE1 inhibitors. Altogether, we proved that APE1 plays a role in the extrinsic cell senescence onset through different mechanisms and that the inhibition of APE1 activity is a promising target for the development of new senolytic compounds.

Lipid-mediated reprogramming of macrophages in early gut carcinogenesis

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Background
The role of intestinal macrophages in the maintenance of mucosa tolerogenicity is often subverted in tumor microenvironment, resulting in their polarization towards immunosuppressive M2-like phenotype. In this context, dysregulation of lipid metabolism has gained an emerging role in pro- tumoral myeloid cell switching, and lipid droplets accumulation has been recently observed in tumor-associated macrophages (TAM) of colorectal cancer (CRC). However, the role of dyslipidemic myeloid cells together with their altered phenotype and immunosuppressive behavior in the early colon carcinogenesis is still unknown.

Results
Here, we report the presence of a subset of CD68bright CD163dim cells, displaying a large morphology with optically empty vacuoles-rich cytoplasm, in human colonic polyps of patients bearing APC-driven polyposis and sporadic adenomas. Present in small aggregates, these cells were mainly localized in polyps-adjacent mucosa within lamina propria. Histological staining showed a weak positivity for acid but not for neutral mucins, while a remarkable positivity for neutral lipids revealed their lipid-engulfed nature. Further, these macrophages subset expresses, HLA-DR, CD36 and PD-L1, as revealed by immunohistochemistry.
By generating an *in vitro* model of lipid-laden macrophages, derived from ex vivo polarization of circulating CD14+ cells from healthy donors, we found that dysregulation of lipid metabolism induced significant changes in expression of HLA-DR, PD-L1, together with CD206 and CX3CR1. Lipid-laden macrophages also affected T cell proliferation, and up-regulated PD-1 expression in proliferative CD4+ and CD8+ subsets compared to the non-foamy counterpart. Of note, an increase of Foxp3 in CD4+ CD25+ CD127- revealed that lipid-engulfed macrophages induce a regulatory T- cells phenotype.

Discussion
The exacerbated internalization of modified lipoprotein significantly affects macrophage in reprogramming their functional polarization. In the context of gut polyposis, the differences between mucosa and adenomas in the presence of lipid-engulfed macrophages could suppress T cell adaptive response and help transformed

epithelial cells in escaping antitumor immunosurveillance. This suggest that these cells may provide novel targets for polyp prevention for the identification of new preventive tool for reducing the early stage of polyp formation.

Non-canonical role of PDK1 as a negative regulator of apoptosis through macromolecular complexes assembly at the ER-mitochondria interface in oncogene-driven NSCLC

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Here we tested whether co-targeting of glucose metabolism and oncogene drivers may enhance tumor response to tyrosine kinase inhibitors (TKIs) in NSCLC.To this end,pyruvate dehydrogenase kinase 1 (PDK1) was stably downregulated in oncogene-driven NSCLC cell lines exposed or not to TKIs. H1993 and H1975 cells were stably transfected with scrambled (shCTRL) or PDK1-targeted (shPDK1) shRNA and then treated with MET inhibitor crizotinib (1 μmol/L), double mutant EGFRL858R/T790M inhibitor WZ4002 (1 μmol/L) or vehicle for 48 h.Effects of PDK1 knockdown on glucose metabolism and apoptosis were evaluated in untreated and TKI-treated cells.PDK1 knockdown alone did not cause significant changes in glycolytic cascade, ATP production and glucose consumption but it enhanced maximal respiration in shPDK1 cells as compared to controls.When combined with TKI treatment, PDK1 downregulation caused a strong enhancement of OXPHOS and a marked reduction of key glycolytic enzymes. Furthermore, increased levels of apoptotic markers were found in shPDK1 cells as compared to shCTRL cells after treatment with TKIs.Co-immunoprecipitation studies showed that PDK1 interacts with PKM2, Bcl-2 and Bcl-xL forming macromolecular complexes at the ER-mitochondria interface.Our findings showed that downregulation of PDK1 is able to potentiate the effects of TKIs through the disruption of macromolecular complexes involving PKM2, Bcl-2 and Bcl-xL.

Plasma microRNAs allow prediction and follow-up of abiraterone acetate efficacy in metastatic castration-resistant prostate cancer patients.

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Abiraterone acetate is still one of the main medications for metastatic castration-resistant prostate cancer (mCRPC). However, since it differs in efficacy from patient to patient, biomarkers are needed for deciding who will be likely to benefit from the treatment. We investigated whether circulating miRNAs are able to stratify patients according to their responsiveness to abiraterone acetate. We analysed plasma from a cohort of mCRPC patients, before and after three, six and nine months abiraterone acetate administration, using miRNA RT-qPCR panels to find miRNAs candidates, and TaqMan RT-qPCR for validation. We found that up-regulation of miR-103a-3p and down-regulation of miR-378a-5p discriminate non-respondent from respondent patients and follow the efficacy of the drug in time. We also explored targets and promoters of the candidates via bioinformatic analysis. Interestingly, HOXB13, described in the literature to be up-regulated in non-respondent patients, is a putative transcription factor of miR-103a-3p, while PTEN, reported to be down-regulated in non-respondent patients is a validated target of the same miRNA. We also showed that LnCaP cells, transfected with mimics and inhibitors of miR-103a-3p and miR-378a-5p respectively, showed a dampened response to abiraterone acetate treatment.
Moreover, an innovative optical sensing technology for the direct detection of the miRNAs in biofluids has been implemented. It relies on sensitive solid-state sensors coupled to a selective chemical method, with no extraction and pre-labelling of the target from biological sources, overcoming the more time consuming and error-prone indirect techniques.

In conclusion, we found two circulating miRNAs predicting abiraterone acetate efficacy in mCRPC patients, and we are implementing a device for their direct measurement, providing new insights on the decision-making for metastatic castration-resistant prostate cancer treatment.

Identification of an epigenetic mechanism that controls genomic stability and epigenetic plasticity and protects from premature cellular senescence.

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Cellular senescence is a permanent state of replicative arrest characterized by increased genome instability and a deep subversion of the transcriptome and of the proteome. A plethora of epigenetic modifications support these changes, but the identification of a unique epigenetic regulator involved in the integration of the different responses is still missing.
Here by means of an unbiased screening we identified HDAC4 as the key epigenetic regulator involved in controlling genome stability and in stemming the transcriptional responses typical of cellular senescence. In particular, we found that HDAC4 is proteasomal degraded during the onset of replicative and oncogene- induced senescence and is progressively degraded in aged mice. The forced depletion of HDAC4 results in the rapid accumulation of double stranded DNA damage and in the activation of enhancers and super- enhancers typical of cellular senescence and leads to premature cell cycle arrest.
From a molecular point of view, HDAC4 controls the rates of H2BK120 acetylation/ubiquitylation by buffering the activity of SAGA complex and promoting the recruitment of HR directed repair complex on genomic loci subjected to fragility and replicative stress. HDAC4 loss not only slows the recruitment of RAD51 and BRCA1 into genomic sites subjected to double-stranded DNA breaks but also unlocks the AP-1/p300 epigenetic program leading to the organization of pro-inflammatory super-enhancers.
Our work highlights the existence of an epigenetic sensor that directly couples the failure in repairing double- stranded DNA breaks to the activation of a secondary epigenetic response that triggers and amplifies cellular senescence.

Castration-induced SPARC down-regulation in stromal cells drives neuroendocrine differentiation of prostate cancer

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Neuroendocrine differentiation (NED) often occurs in a relevant subset of prostate cancer patients as a mechanism of resistance to androgen deprivation therapy (ADT). Tumor microenvironment and its important mediators can sustain tumor cell plasticity toward NED. Among extracellular matrix proteins, secreted protein acidic and rich in cysteine (SPARC) has been deeply studied in different cancer settings exerting different functions when it is produced by the neoplasm or by the neighboring stroma. Here, we elucidated the contribution of stromal SPARC in the onset of NED.
We modeled human disease using TRAMP mice, which spontaneously develop autochthonous prostate tumors following the onset of puberty. Crossing TRAMP mice with Sparc-/- mice, we observed the appearance of focal areas of NED. Interestingly, the same percentage of NED was also observed in TRAMP mice after castration, but not in untreated counterparts, suggesting that SPARC deficiency and castration converge to the same disease outcome. Accordingly, we found that SPARC expression in stromal cells was strongly reduced in castrated TRAMP mice. Moreover, prostate adenocarcinoma cell lines acquired NE features only when cultured in presence of Sparc-deficient fibroblasts or when injected in Sparc-/- mice. This transition occurs through IL-6 release by Sparc-deficient fibroblasts. Indeed, we found that IL-6R targeting limited NED both *in vitro* and *in vivo*. We further detailed a tumor-stroma crosstalk triggered by castration in TRAMP mice that we mimicked *in vitro* by culturing tumor cells and fibroblasts in presence of enzalutamide, a second line generation of ADT drugs. Importantly, we identified the heat shock protein GRP78 as the primer factor of this crosstalk leading to NED through stro-

mal-SPARC down-regulation induced by miR29b released by the tumor. Accordingly, GRP78 targeting strongly reduced NED *in vitro* and *in vivo*. Notably, GRP78 is amplified in human NEPC and its expression is correlated with pathways related to NED. Finally, we also validated this mechanism in a small cohort of patients and, particularly, in paired prostate cancer samples collected before and after ADT, confirming GRP78 and SPARC modulation and the focal up regulation of NE markers.
Data collected so far indicate that stromal SPARC down-regulation skews prostate carcinogenesis toward NED. Our results highlight the pivotal role of stromal components in NED onset, indicating possible diagnostic and therapeutic targets.

The lncRNA TERRA and the hnRNP RALY: insights on a novel functional interaction

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Telomeres prevent the loss of genetic information during DNA replication and the recognition of chromosome ends as the extremities of double strand breaks with the consequent activation of DNA damage response cascades. For these reasons, telomere maintenance mechanisms are essential for preserving genome integrity. Despite their heterochromatic nature, in numerous species telomeres are transcribed, giving rise to a series of long non-coding RNAs, among which TERRA is the best known and studied. TERRA molecules contain subtelomeric-derived sequences at their 5' end and telomeric sequences (UUAGGG in vertebrates) at their 3' extremity. TERRA has been implicated in different aspects of telomere biology, including the maintenance of their epigenetic landscape, the activity of the telomerase enzyme and the safeguard of telomere homeostasis. However, the molecular details of TERRA functions and biogenesis remain to be defined.
RALY is a member of the RNA-binding protein heterogeneous nuclear ribonucleoproteins (hnRNPs) family, which can also associate with DNA. Indeed, RALY has been shown to interact with various promoters and it specifically binds U-rich regions present at the 3' end of RNAs. RALY has been proposed to participate in several biological processes, including splicing and transcriptional regulation; still, its functions are not yet well understood. Recently, RALY was identified as a component of the TERRA interactome in mouse embryonic stem cells (mESC).
We set out to study the interplay between TERRA and RALY in human cancer cells. By using RALY knock-out cells and RALY-targeting gene knockdown approaches we observed that RALY influences both TERRA levels and TERRA dynamics. Furthermore, RALY interacts with TERRA in RNA immunoprecipitation experiments performed on cancer cell lines. Interestingly, our preliminary results indicate that the downregulation of RALY impacts a specific subpopulation of TERRA transcripts, altering TERRA expression levels in a telomere-specific manner. Depletion of RALY associates with an increased number of telomere dysfunction-induced DNA damage foci (TIFs). Overall, our findings point to the TERRA-RALY interaction as an important element for the maintenance of telomeres.

A reversible shift of driver dependence from EGFR to Notch1 in non-small cell lung cancer as a cause of resistance to tyrosine kinase inhibitors

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Background
Notch1 plays a key role in epithelial-mesenchymal transition (EMT) and in the maintenance of cancer stem cells. In the present study we tested whether high levels of activated Notch1

in oncogene-driven NSCLC can induce a reversible shift of driver dependence from EGFR to Notch1 thus causing resistance to EGFR inhibitors. Methods: Adherent cells (parental) and tumor spheres (TS) from NSCLC H1975 cells and patient-derived CD133-positive cells were tested for EGFR and Notch1 signaling cascade. The Notch1-dependent modulation of EGFR, NCID, Hes1, p53 and Sp1 were then analyzed in parental cells by binding assays with a Notch1 agonist DLL4. Results: TS were more resistant than parental cells to EGFR inhibitors. A strong upregulation of Notch1 and a concomitant downregulation of EGFR was observed in TS as compared to parental cells. Parental cell exposure to DLL4 showed a dose-dependent decrease of EGFR and a simultaneous increase of NCID, Hes1, p53 and Sp1 along with the dislocation of Sp1 from the EGFR promoter. Furthermore, an enhanced interaction between p53 and Sp1 was observed in TS. Conclusions: In NSCLC cells, high levels of active Notch1 can promote a reversible shift of driver dependence from EGFR to Notch1, leading to resistance to EGFR inhibitors.

Keywords
Notch1, EGFR, EMT, tumor spheres, NSCLC

Triggering proteotoxic stress in cancer cells: new opportunities for alternative therapies

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Leiomyosarcomas (LMS) are rare but aggressive smooth muscle tumors characterized by complex karyotypes. Limited therapeutic options are available for LMS, thus new therapeutic strategies need to be found. The small molecule 2c is a dienone derivative with two sterically accessible electrophilic ß-carbons that can act as Michael acceptors for target nucleophiles, such as cysteines. 2c triggers multiple stresses, which converge in proteotoxic stress activation. Bioinformatic analysis of a signature of genes upregulated after 2c treatment, involving several elements of the proteotoxic response, correlates negatively with the survival of LMS patients. From this observation, we hypothesize that aggressive LMS coexist with high levels of proteotoxic stress and that these types of cells may reach a limit when challenged with further proteotoxic stress, making them more vulnerable. We show that 2c can induce proteotoxic stress in LMS cells. Indeed, the chaperones HSPA6 and HSPA1A show a dramatic increase in mRNA levels in these cells after treatment with 2c. Moreover, 2c triggers mitochondrial dysfunction and by STED technique microscopy we unveil that this small molecule can reorganize the sub-mitochondrial clusters of DIABLO/SMAC. To improve its efficiency in vivo, 2c was engineered through a conjugation with PEG and a small peptide, generating a pro-drug version of the compound called 2cPP. This new molecule can release 2c through the action of secreted proteases present in the tumor microenvironment. 2cPP induces a similar level of cell death in LMS cells as 2c, but unlike 2c, is unable to induce cell death in normal smooth muscle cells. When assessed for anti-tumoral activities in vivo, using different xenograft models of LMS, 2cPP showed a strong anti-tumor effect. All our data seem promising for LMS treatment and highlight that proteotoxic stress may be an alternative strategy in anti-cancer therapy.

Microfluidic-based platform for automated aging studies using the nematode C. elegans.

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Caenorhabditis elegans (C. *elegans*) has been used extensively over the last half- century to address fundamental aspects of biological research, such as the mechanisms of aging and neurodegeneration. Indeed, this small nematode can serve as an ideal model for the study of aging due to its short lifespan, tractable genetics and the conservation of essential age-related metabolic pathways between C. *elegans* and

humans. Over the last decade, significant progress has been made in developing new methods for studying C. *elegans*. However, there is still a lack of an "all-in-one" solution providing full automation and standardization of all the key aspects of C. *elegans* experimentation, including culture, genetic treatment, observation, as well as data recording and analysis. Moreover, monitoring the dynamics of aging processes (e.g. gene and protein expression, reproductive aging, motility, etc.) over time would be a valuable tool, but it remains a standing challenge of traditional C. *elegans* methods. Recently, it has been demonstrated that various aspects of C. *elegans* research can be improved using microfluidic technologies. In this context, we developed a new microfluidic platform allowing full automation of the entire process of culture, treatment, imaging and analysis of C. *elegans*, at unprecedented levels of throughput and standardization. The unique possibilities offered by this platform allow the automated execution of customized bioassays, specifically tailored to the investigation of C. *elegans* aging and age-related processes. Here, we present a set of bioassays for: (i) monitoring C. *elegans* lifespan and healthspan; (ii) quantifying the GFP expression of reporter strains; (iii) identifying developmental phenotypes that can serve as potential predictors of aging. In particular, we employ the hsp-6:gfp transgenic strain as a specific reporter for the mitochondrial unfolded protein response (UPRmt). By treating hsp-6::gfp worms with the antibiotic doxycycline, we can activate the UPRmt and efficiently quantify its level by measuring the fluorescence intensity of the worms. Finally, to test the potential of our lifespan and healthspan bioassay, we expose wild-type worms to different dietary restriction plans. In this study, we show that the platform allows the detection of early phenotypes related to the aging process, such as reproductive aging and motility, and we successfully demonstrate the extended lifespan of C. *elegans* as a result of precise dietary restriction plans in our system.

The cyclin-dependent kinase inhibitor p21 mediates ERK5-dependent cellular senescence in melanoma cells

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The Mitogen Activated Protein Kinase ERK5 supports cell proliferation in human melanoma. Melanoma is frequently characterized by the loss or the reduced expression of molecules involved in the promotion of cellular senescence, a permanent state of cell cycle arrest, that would restrain tumor growth. To deepen the role of ERK5 in melanoma growth, we performed transcriptomic analysis following ERK5 knock-down (KD) in BRAF-mutated melanoma cells and found that cellular senescence was among the most affected processes. Because pro-senescence drugs are actively sought after for cancer treatment, in this work we evaluated the effects of the inhibition of ERK5 on cellular senescence in melanoma cells, in order to find a valid approach against tumor growth and progression.
In human melanoma cell lines (A375, Sk-mel-5 and SSM2C), expressing either wild type or oncogenic (V600E) BRAF, ERK5 targeting was achieved using small-molecule inhibitor XMD8-92 (5 μM) or ERK5-specific shRNA. Cellular senescence was evaluated by senescence associated ß-Galactosidase assay, followed by subsequent analysis in bright field microscopy. Furthermore, genetic inhibition of p21 was performed on A375 cells with two p21-specific shRNA (shp21-1 and shp21-2). The expression of p21 was evaluated by western blotting analysis on cell lysates. Parental and p21-KD A375 cells were treated with DMSO or with XMD8-92 (5 μM) for 72 hours. Treatment with 300 nM H2O2 during the first 2 hours was used as a positive control. The percentage of SA-ßGal positive cells (blue ones) was then quantified.
We found that both genetic and pharmacological inhibition of ERK5 elicited cellular senescence, as witnessed by the marked increase of senescence associated ß-galactosidase activity and of p21 expression. Moreover, our data reveals that p21 KD halved the percentage of cells undergoing ERK5-related cellular senescence following treatment with XMD8-92 or H2O2, pointing to p21 as a key mediator of this process.
Based on our results, small-molecule compounds targeting ERK5 emerge as pro-senescence drugs that may be exploited for melanoma treatment. Furthermore, the above data indicate that cellular senescence induced by ERK5 inhibition in melanoma cells relies, at least in part, on p21.

γ-Radiation-induced senescence from DNA damage to inflammatory pro-tumoral phenotype: protective effects of olive phenols in human fibroblasts.

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Senescence occurs upon critical telomere shortening (replicative senescence), or following DNA damage, oncogenic activation, hypoxia and oxidative stress, overall referred to as Stress-Induced Premature Senescence (SIPS). Senescent cells do not divide, though remaining metabolically active. In response to DNA damage, they release cytoplasmic chromatin fragments (CCFs), and are known to express an altered secretome, the Senescence-Associated Secretory Phenotype (SASP), which contributes to generate a pro-inflammatory and pro-tumoral extracellular milieu. Polyphenols have gained significant attention owing to their anti-inflammatory and anti-tumor activities. Here, we studied the effect of oleuropein aglycone (OLE) and hydroxytyrosol (HT) on DNA damage, CCF appearance and associated intracellular pathways, and SASP in a model of irradiation-induced senescence. Neonatal human dermal fibroblasts (NHDFs) were γ-irradiated and incubated with OLE, 5μM, and HT, 1μM. Cell growth and Senescence-Associated (SA)-β-Gal staining were used as senescence markers. DNA damage and nuclear stability were evaluated by Comet assay, Lamin B1 expression, release of CCFs, cyclic GMP-AMP Synthase (c-GAS) activation. Fibroblast-conditioned media were analyzed for IL-6, IL-8, MCP-1 and RANTES by ELISA assay. Our results showed that 8 Gy irradiation was effective in inducing premature senescence, and that OLE and HT exerted a protective effect, preserving Lamin B1 expression and reducing CCFs. We also demonstrated a reduction of c-GAS/Stimulator of Interferon Genes (STING) activation, NFkB nuclear localization and SASP factor release. The ability of OLE and HT to mitigate DNA damage, senescence status and the related SASP in normal cells can be exploited to improve the efficacy and safety of cancer radiotherapy.

Peptide targeting of lysophosphatidylinositol-sensing GPR55 for osteoclastogenesis tuning

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The G-protein-coupled receptor GPR55 has been implicated in multiple biological activities, which has fuelled interest in its functional targeting. Its controversial pharmacology and often species-dependent regulation have impacted upon the potential translation of preclinical data involving GPR55. With the aim to identify novel GPR55 regulators, we have investigated lysophosphatidylinositol (LPI)-induced GPR55-mediated signal transduction. The expression system for wild-type and mutated GPR55 was HeLa cells silenced for their endogenous receptor by stable expression of a short-hairpin RNA specific for GPR55 5'-UTR, which allowed definition of the requirement of GPR55 Lys80 for LPI-induced MAPK activation and receptor internalisation. In RAW264.7 macrophages, GPR55 pathways were investigated by Gpr55 silencing using small-interfering RNAs, which demonstrated that LPI increased intracellular Ca2+ levels and induced actin filopodium formation through GPR55 activation. Furthermore, the LPI/GPR55 axis was shown to have an active role in osteoclastogenesis of precursor RAW264.7 cells induced by 'receptor-activator of nuclear factor kappa-ß ligand' (RANKL). Indeed, this differentiation into mature osteoclasts was associated with a 14-fold increase in Gpr55 mRNA levels. Moreover, GPR55 silencing and antagonism impaired RANKL-induced transcription of the osteoclastogenesis markers: 'nuclear factor of activated T-cells, cytoplasmic 1', matrix metalloproteinase-9, cathepsin-K, tartrate- resistant acid phosphatase, and the calcitonin receptor, as evaluated by real-time PCR. Phage display was previously used to identify peptides that bind to GPR55. Here, the GPR55-specific peptide-P1 strongly inhibited osteoclast maturation of RAW264.7 macrophages, confirming its activity as a blocker of GPR55-mediated functions. Although osteoclast syncytium formation was not affected by pharmacological regulation of GPR55, osteoclast activity was dependent on GPR55 signalling, as shown with resorption assays on bone slices, where LPI stimulated and GPR55 antagonists inhibited bone erosion. Our data indicate that GPR55 represents a target for development of novel therapeutic approaches for treatment of pathological conditions caused by osteoclast-exacerbated bone degradation, such as in osteoporosis or during establishment of bone metastases.

Evaluation of a series of pimeloylanilide o-aminoanilide (PAOA) derivatives as inhibitors of the MEF2-HDAC axis in leiomyosarcomas

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Class IIa HDACs are a group of proteins that have an important role in the regulation of epigenetic and transcriptional context in the cell. HDACs IIa are able to carry out this regulation through the binding with some transcriptional factors at the level of their extended N-terminal region. One of the main partners of HDACs IIa is the family of transcription factors MEF2, which coordinate many differentiative and adaptive responses. Dysregulation of the MEF2-HDACs IIa axis has been observed in some tumoral contexts, such as in leiomyosarcomas (LMS), where there is simultaneous upregulation of MEF2 factors and HDACs IIa proteins. LMS are very rare and aggressive tumors and nowadays there are no effective therapies. In this work, we investigated the possibility of targeting the binding between MEF2 and HDACs as a potential treatment for LMS. We used a series of small molecules, mainly PAOA derivatives, which are predicted to bind the hydrophobic groove present in the MADS -box/MEF2 domain of MEF2. Competitive binding of these compounds should unleash the interaction with class IIa HDACs, thereby restoring the transcriptional activity. The pan-HDACi SAHA (Vorinostat) and the class IIa HDACi TMP-195 were used for comparison. We observed that some of these PAOA derivatives selectively induced apoptosis in tumor cells. However, in a fluorescent polarization assay in vitro and in co-ip experiments in vivo, they were unable to disrupt the interaction between MEF2A/D and HDAC4. In vivo studies proved that these PAOA derivatives act as "classical" HDAC inhibitors. Comparative transcriptome analyzes confirmed that, like SAHA, the PAOA derivatives interfere with cellular responses. Interestingly, the expression of several class IIa HDACs and MEF2 members is modulated by SAHA and PAOA derivatives, suggesting that an effect on the MEF2-HDAC axis may be indirect. Importantly, the expression of a panel of chemokines is modulated jointly by TMP195 and by the MEF2A-HDAC axis. This finding suggests that specific class IIa HDACs inhibitors should be evaluated to potentiate immunotherapy against LMS.

The SHERPA project: SHP2 and ERK inhibitors as a novel strategy for treating Pancreatic cancer.

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Almost every patient diagnosed with pancreatic ductal adenocarcinoma (PDAC) harbors a mutation in the KRAS oncogene. KRAS signals to the MAPK pathway leading to increased proliferation, but can also activate the PI3K/AKT/mTOR axis promoting cell growth and cell survival. Since KRAS is undruggable (with the exception of G12C mutation), inhibition of downstream KRAS effectors using MEK or ERK inhibitors has been tested in the clinic. Unfortunately, tumor cells rapidly acquire resistance to these single targeted therapies. In our laboratory and others, it has been demonstrated that resistance often occurs through feedback activation of Receptor Tyrosine Kinases (RTK's) resulting in restoration of MAPK signaling. SHP2, is a targetable phosphatase acting downstream of most RTKs and upstream of KRAS. In this project, we combine SHP2 and ERK inhibitors in several PDAC models, both *in vitro* and *in vivo*. We observe full MAPK inhibition and induction of apoptosis when using this drug combination. Importantly, the combination is well-tolerated *in vivo*, hence it is promising for testing in the clinic. Moreover, we performed a genome-wide CRISPR screen to identify possible emerging resistance mechanisms to SHP2 + ERK co-inhibition. We

discovered that PTEN knockout can induce cell survival and proliferation upon administration of both inhibitors. Similarly, spontaneous resistors, although difficult to obtain, also appear to be driven by PTEN downregulation. Accordingly, adding a mTOR inhibitor to the SHP2 + ERK inhibitor combination prevents the emergence of resistance in a dose-dependent manner. Our results show that combined targeting of SHP2 and ERK holds significant promise for treating KRAS mutant PDAC tumors. Our data also indicate that PI3K/AKT/mTOR pathway activation may serve as a marker for sensitivity/resistance to this combination.

Precision Revisited: Targeting Microcephaly genes in Brain Tumors

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Medulloblastoma (MB) is the most frequent high-grade brain tumor in children. The standard treatment for these tumors consists in surgery, followed by radiotherapy and chemotherapy. Despite the improvement in patient survival, these therapies are only partially effective. Many patients still die and those who survive suffer from neurological and endocrine disorders. Therefore, more effective therapies are needed Primary microcephaly (MCPH) is a rare disorder caused by mutations in 20 different genes, specifically implicated in the developmental expansion of neural progenitors. Centromere-associated protein E (CENPE) heterozygous mutations cause the MCPH13 syndrome. CENPE is a microtubule plus-end- directed kinetochore motor protein important in chromosome congression, spindle microtubule capture at kinetochores and spindle checkpoint activation. As for other MCPH genes, CENPE is required for normal proliferation and survival of neural progenitors, but has limited effects on other tissues. Since there are evidences that MB shares many molecular features with neural progenitors, we hypothesized that CENPE could be an effective target for MB treatment. In ONS-76 and DAOY, CENPE knockdown increases both mitosis defect and apoptosis. Indeed, CENPE's siRNA prolongs cells metaphase inducing mitotic catastrophe via p53 or p73 signaling pathway. To consolidate CENPE as a target for MB treatment, we tested GSK923295, an allosteric inhibitor that binds the ATPase pocket already in clinical trial for other cancer types. GSK923295 induces same effect of knockdown, but with higher penetrance, at nM level in MB cell lines. These results suggest that CENPE's chemical inhibition could be a useful target for MB treatment.

HAS2-AS1 Overexpression mediates apoptosis induction in MDA-MB- 231 triple negative breast cancer cells

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Although several studies report that hyaluronic acid (HA) favours tumour growth and spreading *(Heldin P et al. J Biochem. 2013)*, the molecular mechanisms behind this cancer-promoting effect are not fully clear.
We recently described that the natural antisense transcript HAS2-AS1, a long non-coding RNA, is critical to control hyaluronan synthase 2 (HAS2) gene transcription via epigenetic modifications in aortic smooth muscle cells *(Caon I et al. J Biol Chem. 2020)*. Yet, this mechanism does not apply to the triple-negative breast cancer (TNBC) cell line MDA-MB-231, in which HAS2 expression levels remain unaltered after overexpressing or silencing HAS2- AS1, as well as secreted and pericellular HA levels.
We found that TNBC cell lines express higher levels of HAS2-AS1 rather than ER/PR positive cell lines. Surprisingly, we demonstrated that HAS2-AS1 overexpression in TNBC cell line MDA-MB-231 diminished significantly cell survival, migration, and invasion compared to wild-type cells. Conversely, HAS2-AS1 knockdown stimulated a malignant phenotype, increasing cell survival, migration, and invasion.
An intriguing cytoplasmic function of lncRNAs is their ability to bind miRNAs, creating a competition for the interaction between miRNA, lncRNA and other regulatory targets (ceRNAs).
In silico analysis revealed that HAS2-AS1 exon 2 transcript contains several putative binding sites for different miRNAs, among which miR186-3p. Indeed, luciferase assays confirmed the interaction between HAS2-AS1 and miR186-3p.
Microarray analysis on HAS2-AS1 silenced MDA-MB-231 cells revealed an altered gene profile related to several pathways critical for tumour progression, including apoptosis, transport, autophagy, and EMT.
By TUNEL assay, Western blotting and measuring the activity of caspases 3 and 7, we observed an increment of apoptosis after HAS2-AS1 overexpression in MDA-MB-231 cells with respect to the control sample, likely via the purinergic receptor P2RX7 *(Zhou L et al., J Biol Chem. 2008)*.
In conclusion, all these data suggest that HAS2-AS1 abrogation increased aggressiveness whereas HAS2-AS1 overexpression reduced aggressive-

ness in the triple-negative MDA- MB231 cell line. Moreover, the sponge effect of HAS2-AS1 can antagonise the function of miRNA186-3p on its downstream targets inducing, among all, apoptotic pathway.

Deciphering common mechanisms between senescence and tumor dormancy

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Every tumor is highly heterogeneous: cancer cells interact to support its growth, invasion and immune escape. An important feature of cancer cells is their ability to establish a state called "dormancy", which resembles some of the characteristics described for the state of quiescence and senescence. Dormant cells are able to circulate throughout the body, seed tissues, stay quiescent for long periods of time, and eventually re-awaken.
The definition of dormancy is still controversial, even though in the last years it has been proposed that different mechanisms are able to promote different dormant states. Regardless of their definition, little is known about the mechanisms that are able to re-awake these cells.
Another type of cell cycle arrest involved in cancer is senescence. Senescence was first described as a tumor-protective mechanism, while later in the years a tumor supporting role has also been highlighted.
Both dormancy and senescence describe a proliferative arrest: while senescence is usually described as irreversible, dormancy is a highly reversible condition.
A big debate in the dormancy field focuses on the role of senescence during tumor dormancy. Many questions are still open, such as the similarities between dormancy and senescence and the interaction between these cells in order to induce cancer relapse.
Another cell cycle arrest described in nature is "diapause": diapause is a physiological reproductive strategy widely employed across species, including mammals, characterized by a reversible state of suspended development triggered by unfavorable conditions. The diapause state of embryonic stem cells (ESCs) shows some similarities with the dormant state of cancer cells.
We have established an in vitro system to reproduce tumor dormancy based on an approach developed by Bulut-Karsioglu in 2016 for the induction of diapause in ESCs.
As we would like to answer some of the questions regarding the relationship between dormant and senescent cancer cells, we are using this approach to characterize the dormant phenotype in vitro in comparison with the senescent phenotype.
Our aim is to find some common vulnerabilities and to understand the crosstalk between these different proliferative arrests. In order to achieve these, we are also performing a Crispr/Cas9 screening to find genes that are essential for the survival of dormant cancer cells.

Is the switching on of endogenous retroviral elements necessary and sufficient to sustain senescence and aging?

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Cellular responses to RNA and DNA virus infection involve specific classes of host pattern recognition receptors (PRRs) of innate immune system, that detect invading viral RNAs and DNAs and supervise different subcellular compartments. These nucleic acid sensors include the endosomal Toll- like receptors (TLRs 3,7,8,9), the cytosolic DNA sensor cyclic GMP–AMP synthase (cGAS) and the cytoplasmic RNA sensor retinoic acid- inducible gene I (RIG- I)- like receptors (RLRs).The latter class that comprise mainly RIG- I and melanoma differentiation- associated protein 5 (MDA5) act as dsRNA sensor. In RLR signal pathway, a common adaptor protein is mitochondrial antiviral- signalling protein (MAVS) that finally leads to a potent antiviral response. The activation of these sensors induces proliferation arrest, senescence and apoptosis. In different models of cellular senescence, we found a cytoplasmic accumulation of DNA derived from the B1 nuclear lamina dismantling and accumulation of DNA damage. On the other hand, in the same cellular models, we observed increased levels of ERVs (endogenous retrovirus) transcripts mainly folded as dsRNA; interestingly, dsRNA levels are unrelated to genome instability. The derepression of ERVs is due to the H3K27me3 demethylation. Curiously this epigenetic response is achieved after the knockout of Histone deacetylase 4 (HDAC4), a member of the class IIa of HDAC. dsRNAs accumulation and the subsequent activation of IFN response contribute to enhance the inflammatory microenviroment. Similarly, the exogenous delivery of dsRNAs immunopurified from HDAC4-/- cells or the transfection of equimolar amounts of POLY:IC, synthetic molecules mimicking dsRNAs, is able to trigger senescence and cell death in low

grade sarcoma cells and in normal fibroblasts.
With our work we aim to better understand if the ERVs deregulation and in turn the activation of the antiviral response is essential to promote and sustain the senescence program or if it represent a secondary response in this process. To this purpose we are generating cellular inducible models of senescence in which we will knock-down the expression of RIG-1, MAVS and MDA5.
We are confident that our work will discolse a new pathway of regulation of the pro-inflammatory environment involved in sustaining and/or restraing senescence and aging.

Therapy induced senescent stroma increases prostate cancer aggressiveness

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Docetaxel represents the gold-standard treatment option for metastatic castrate resistant prostate cancer (mCRPC). However, many patients fail to respond to the therapy, ultimately experiencing chemotherapy insensitivity and cancer relapse. Thus, dissecting the molecular basis that correlate chemotherapy with its long-term adverse effects is an extremely urgent clinical need. Cellular senescence has recently emerged as a tumor promoting mechanism due primarily to the senescence-associated secretory phenotype (SASP), characterized by the secretion of a plethora of pro-inflammatory cytokines, growth factors and matrix metalloproteinases that strongly contribute to create a tumor permissive microenvironment. Remarkably, a recent body of evidence underlined that anticancer drugs promote the senescent phenotype, thereby accounting for many of their by-stander effects. Here, we highlighted that Docetaxel treatment strongly induces the senescent phenotype in stromal prostate fibroblasts, characterized by the enhancement of several senescence markers and different SASP factors as well as by the acquisition of dysfunctional mitochondria and increased ROS production. The clinical importance of these in vitro findings was validated through the analysis of lipofuscin accumulation in tissue specimens from 20 patients affected by prostate cancer, 10 of whom were subjected to Docetaxel therapy before radical prostatectomy. Interestingly, lipofuscin staining has been found increased in the Docetaxel-treated cohort of patients with a preferential localization in the stromal compartment of prostate cancer tissues. Since tumor progression is strongly dependent on the reciprocal cross-talk between cancer cells and the surrounding microenvironment, we then incubated PC3 cells, a metastatic prostate cancer cell line, with conditioned medium isolated from control or Docetaxel- induced senescent fibroblasts. We evidenced that senescent fibroblasts sustain the increase in the migratory, invasive, clonogenic and stemness potential of prostate cancer cells. Interestingly, the treatment of senescent stromal cells with the senolytic drug ABT-263 selectively reduces the number of senescent fibroblasts, ultimately reverting the aggressive phenotype of prostate cancer cells. In conclusion, we underlined that Docetaxel might account for many of its adverse effects, including chemotherapy insensitivity, through its ability to promote the senescent phenotype in the stromal compartment of prostate cancer, thus resulting in increased tumor malignancy. In addition, the so-called "one-two punch strategy", consisting in the sequential administration of a traditional anticancer drug and a senolytic compound, could be proposed for prostate cancer management.

Protein kinase C regulates fatty acid metabolism in breast cancer cells

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Introduction
Lipid metabolism is often modulated in different pathologies including cancer but the molecular regulators of these changes are less understood. In breast cancer cells, protein kinase (PKC) has

been shown to trigger malignant progression. It is conceivable that PKC can contribute to cancer plasticity by regulating lipid metabolism.

Materials and Methods
We used liquid chromatography-mass spectrometry (LC-MS/MS) to identify proteomic differences in breast cancer models after PKC activation. We validated differentially expressed proteins by western blot and biochemical/functional approaches in vitro and in vivo.

Results
The observation that breast cancer models with epithelial and mesenchymal features are characterised by a specific fatty acid metabolism [1] suggests that specific signalling networks are involved in the control of lipogenesis and lipolysis to sustain cancer growth. Here, we demonstrated that PKC activation drives the expression of metabolic enzymes involved in fatty acids mobilization from triacylglycerols (TAG) and lipid utilization in the mitochondria through fatty acid oxidation (FAO). In triple negative cells, we report that PKC regulates intracellular fatty acid metabolism through the up-regulation of N-Myc Downstream Regulated 1 (NDRG1) and acyl-coenzyme A (CoA) synthetase long chain family member 3 (ACSL3). In vivo, NDRG1 is highly expressed in triple-negative breast cancer (TNBC) as compared to estrogen receptor–, progesterone receptor– or human epidermal growth factor 2 receptor–positive breast cancer.

Conclusion
In the study, we identified a proteomic signature that was associated with PKC activation in breast cancer. This is correlated with the modulation of fatty acid metabolism and increased FAO through NDRG1 and ACLS3. Overall, these findings demonstrate the critical role of PKC in supporting the increased bioenergetic reliance on FAO of TNBC.

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Topics of all Pezcoller Foundation Symposia

31. Trento, 17 - 18 June 2019
CANCER AS A CORRUPTED TISSUE

30. Trento, 25 - 26 June 2018
OVERCOMING THE INNATE RESISTANCE OF CANCER TO THERAPY

29. Trento, 22 - 23 June 2017
BUILDING NEW BRIDGES BETWEEN BASIC AND CANCER SCIENCE

28. Trento, 20 - 21 June 2016
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MOLECULAR IN VIVO VISUALISATION OF CANCER CELLS

14. Trento, 30 May - 1 June 2002
THE NOVEL DICHOTOMY OF IMMUNE INTERACTIONS WITH TUMORS

13. Rovereto, 31 May - 2 June 2001
FOCUSING ANALYTICAL TOOLS ON COMPLEXITY IN CANCER

12. Trento, 1 - 3 June 2000
SIGNALING CROSS-TALKS IN CANCER CELLS

11. Rovereto, 5 - 7 June 1999
MOLECULAR HORIZONS IN CANCER THERAPEUTICS

10. Trento, 29 June - 1 July 1998
THE GENETICS OF CANCER SUSCEPTIBILITY

9. Rovereto, 4 - 7 June 1997
THE BIOLOGY OF TUMORS

8. Trento, 17 - 19 June 1996
GENOMIC INSTABILITY AND IMMORTALITY IN CANCER

7. Trento, 14 - 16 June 1995
CANCER GENES. FUNCTIONAL ASPECTS

6. Rovereto: 29 June - 1 July 1994
NORMAL AND MALIGNANT HEMATOPOIESIS: NEW ADVANCES

5. Trento, 9 - 11 June 1993
APOPTOSIS

4. Rovereto, 24 - 26 June 1992
ADHESION MOLECULES: CELLULAR RECOGNITION MECHANISMS

3. Trento, 5 - 7 June 1991
TUMOR SUPPRESSOR GENES

2. Rovereto, 11 - 13 June 1990
THE THERAPEUTIC IMPLICATIONS OF THE MOLECULAR BIOLOGY OF BREAST CANCER

1. Trento, 19 - 21 June 1989
DRUG RESISTANCE: MECHANISMS AND REVERSAL

Pezcoller Foundation – AACR International Award for Extraordinary Achievement in Cancer Research 2021

Award Ceremony

Teatro Sociale, Trento - September 18, 2021

Group leader at the Hubrecht Institute for Developmental Biology and Stem Cell Research and at the Princess Máxima Center for Pediatric Oncology (Utrecht), Professor at the University Utrecht and Oncode Investigator.

Motivation

Clevers is being honored for a series of breakthrough discoveries that led to the development of mini-organs, now called **organoids**, and is widely considered one of the world's leading experts on **adult stem cell biology**.

The ability to generate organoids from stem cells has been an essential first step towards the growth of the regenerative cancer medicine field. This unique cancer model system has also been instrumental in establishing new avenues of research involving the testing of novel anticancer therapeutics on tissues derived from tumors and cultured as organoids.

Early in his career, Clevers' research group first studied **intestine behavior in normal physiological states**.

During these studies, his group cloned the transcription factor TCF1, which has since been proven to be a vital component in the **Wnt signaling pathway**. Next, Clevers demonstrated the link between Wnt signaling and adult stem cell biology by demonstrating that TCF4 gene disruption leads to the abolition of small intestine crypts, while targeted knockout of the TCF1 gene severely disables the stem-cell compartment of the thymus.

Together with Bert Vogelstein, he also showed

that mutations in the Wnt signaling pathway are capable of contributing to **colon cancer onset and progression**. This finding has since propelled countless research efforts focused on the development of novel anticancer therapeutics that precisely target the Wnt signaling pathway. His pioneering research into stem cell biology, which led to the establishment of organoids as an essential model system for cancer research, has deepened our understanding of cancer's origins and **revolutionized cancer drug development** to the great benefit of patients worldwide.

About the Winner:
Clevers earned his medical degree (1984) and doctorate in biology (1985) at the University of Utrecht, the Netherlands.
He is now the principal investigator at the Hubrecht Institute for Developmental Biology and Stem Cell Research, and the principal investigator at the Princess Máxima Center for Pediatric Oncology, Utrecht. Additionally, he is an investigator at the Oncode Institute in the Netherlands, and a professor in Molecular Genetics at the University Medical Center in Utrecht. He received more than 34 national and international Awards, he is author of around 700 scientific research papers, with more than 120.000 citations.

The Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research was assigned to Hans Clevers, MD, PhD, at the virtual AACR Annual Meeting (April 10-15, 2021).

The traditional Award Ceremony in Teatro Sociale, Trento (Italy) will be held in September 18, 2021.

Pezcoller Foundation – AACR International Award for Extraordinary Achievement in Cancer Research

2022 Program guidelines and nomination instructions

NOMINATION DEADLINE
August 31, 2021

NOMINATION PROCESS

Nominations may be submitted by any individual, whether an AACR member or nonmember, who is currently or has previously been affiliated with any institution involved in cancer research, cancer medicine, or cancer-related biomedical science. Self-nominations are prohibited.
Nominators must maintain strict confidentiality of their nominations and all nominations must be submitted online to https://myaacr.aacr.org. Paper nominations will not be accepted.
Eligible nominations must include the following:
• A nomination letter written in English (Max: 1,000 words) that comprehensively describes the nominee's major scientific achievement(s) in basic cancer research and/or their significant contributions to translational cancer research, and the impact of these accomplishments on the field. Letter must specifically outline the candidate's current research activity and indicate how this research holds promise for continued substantive contributions to the cancer field. All publications that directly support the mentioned research accomplishments must be referenced within the provided letter.
• A brief scientific citation (Max: 50 words) highlighting the major scientific contribution(s) justifying the award candidate's nomination.

AWARD ELIGIBILITY CRITERIA
The prestigious Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research was established in 1997 to recognize a scientist of international renown who has made a major scientific discovery in basic cancer research or who has made significant contributions to translational cancer research.
Eligible candidates must continue to be active in cancer research, have a record of recent, noteworthy publications, and be conducting ongoing work that holds promise for continued substantive contributions to progress in the field of cancer.
The Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research is intended to honor an individual scientist. However, more than one scientist may be co-nominated and selected to share the award in the event that their investigations are intimately related in subject matter and have resulted in work that is worthy of the award and a joint nomination.
Cancer researchers affiliated with institutions in academia, industry, or government involved in cancer research, medicine, or cancer-related biomedical science anywhere in the world are eligible. The Award may only be presented to an individual investigator.
Individuals who have previously been awarded the Nobel Prize in any category are ineligible to receive this Award.
Institutions and/or organizations are not eligible to receive the award.

AWARD SELECTION PROCESS
Eligible nominees will be considered by a prestigious Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research Selection Committee consisting of an international cohort of renowned cancer leaders appointed by the AACR President in consultation with the Pezcoller Foundation Council.
The Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research Selection Committee will consider all nominations as they have been submitted and are restricted from combining submitted nominations, adding new nominees, or otherwise making alterations to any submitted nomination.
Once chosen, the primary and alternate award recipient selections made by the Pezcoller Foundation-AACR International Award for Extraordi-

nary Achievement in Cancer Research Selection Committee shall be sent to the AACR Executive Committee and the Pezcoller Foundation Council for final consideration and ratification.Selection of the Award recipient shall be made on the basis of the candidate's scientific accomplishments without regard to race, gender, nationality, geographic location, or religious or political views.

THE AWARD RECIPIENT

The Award recipient will receive an unrestricted grant of € 75,000, a commemorative award, and present a scientific lecture in conjunction with the AACR Annual Meeting immediately following their selection. The Award recipient will also present scientific lectures at the University of Padua and at the University of Trento in Italy, just prior to the official Award ceremony in Trento, Italy in May 2022.

For all information:
https://www.aacr.org/wp-content/uploads/2021/05/2022-Pezcoller-Foundation-AACR-Award-Program-Guidelines-FINAL.pdf

Pezcoller Foundation

EACR Awards 2021

1. Translational Cancer Researcher Award:

ANDREA ABLASSER

Swiss Federal Institute of Technology in Lausanne (EPFL)

2. Women in Cancer Research Award:

KAREN VOUSDEN

Francis Crick Institute, London

3. Rising Star Award:

SAM BEHJATI

Wellcome Sanger Institute, Cambridge

Nominations for the 2022 Awards will open in Autumn 2021:
https://www.eacr.org/pezcoller-foundation-awards

The Pezcoller Foundation

Journal

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