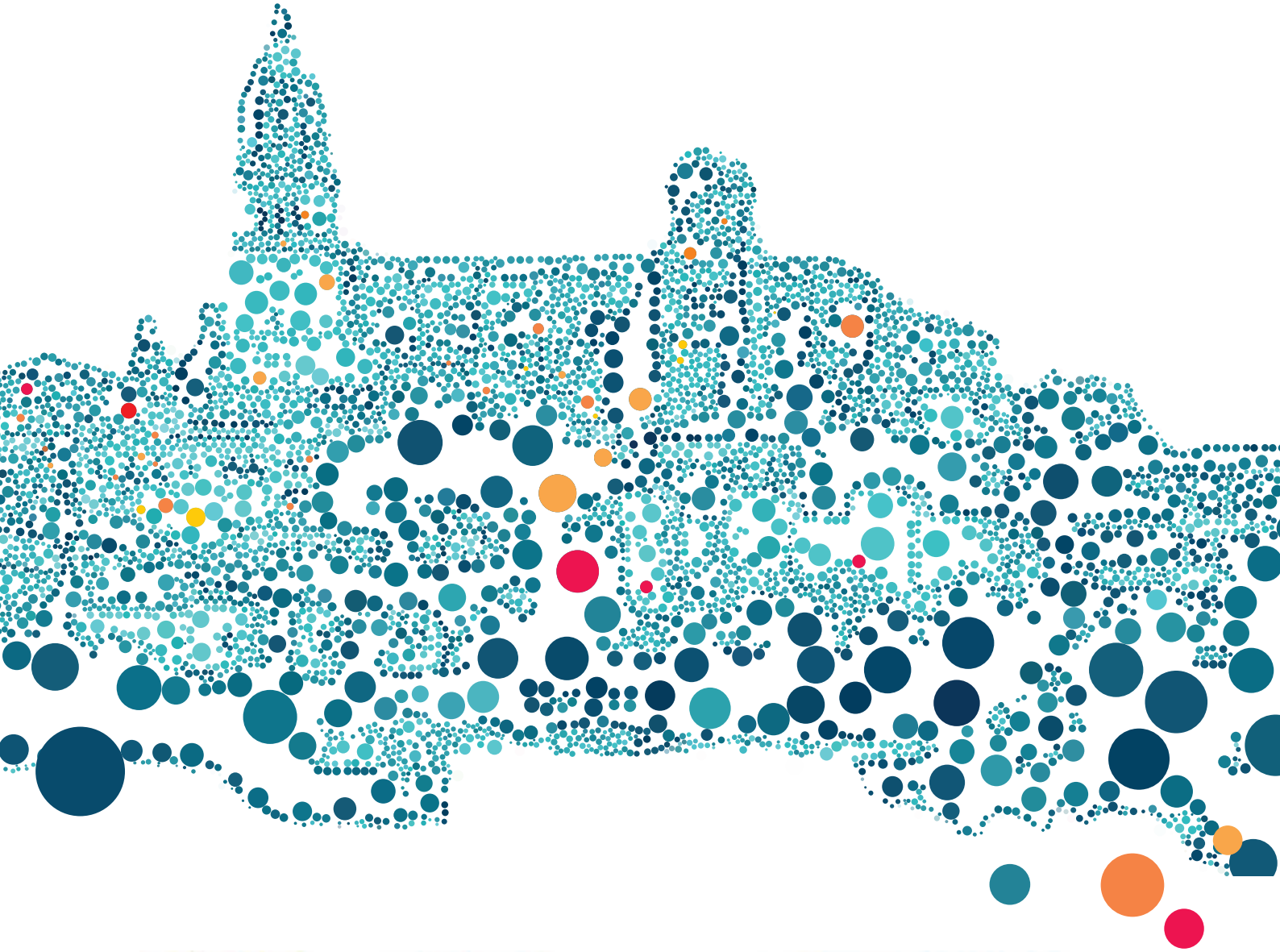
Symposium on

Cancer genomics and epitranscriptomics: from the bench to the clinic

November 30th to December 2nd 2022
(Salamanca, Spain)



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Design and layout: a.f. diseño y comunicación / www.afgrafico.com

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SHORT BIO OF SPEAKERS

Manel Esteller

Director of the Josep Carreras Leukaemia Research Institute (IJC), and ICREA Research Professor Genetics Chairman, School of Medicine, University of Barcelona, Barcelona, Spain

He graduated in Medicine from the Universitat de Barcelona, where he also obtained his Ph.D. in molecular genetics. Dr. Esteller was a Postdoctoral Fellow and a Research Associate at Johns Hopkins where he studied DNA methylation and human cancer. His work was decisive in establishing promoter hypermethylation of tumor suppressor genes as a common hallmark of cancer. From October 2001 to September 2008 Manel Esteller was the Leader of the CNIO Cancer Epigenetics Laboratory, where his principal area of research were the alterations in DNA methylation, histone modifications and chromatin in human cancer. Since October 2008 until May 2019, Dr Esteller has been the Director of the Cancer Epigenetics and Biology Program (PEBC) in Barcelona. He is currently the Director of the Josep Carreras Leukaemia Research Institute (IJC), Chairman of Genetics in the School of Medicine of the University of Barcelona, and an ICREA Research Professor. His current research is devoted to the establishment of the epigenome maps in health and disease, and the development of new epigenetic drugs. Author of numerous and highly cited peer-reviewed manuscripts in biomedical sciences, he is also a Member of numerous international scientific societies, Editorial Boards and reviewer for many journals and funding agencies. He has received prestigious recognitions for his scientific achievements among them the World Health Summit Award, the Swiss Bridge Cancer Award and the EACR Cancer Researcher Award Lecture.

Mathieu Lupien

Senior Scientist at the Princess Margaret Cancer Centre and Professor at the University of Toronto. Cross-appointment with the Ontario Institute for Cancer Research, Toronto, Canada

Dr. Lupien's research in chromatin & epigenetics has pioneered the study of the non-coding genome to identify the genetic determinants of oncogenesis and accelerate the development of chromatin & epigenetic-based precision medicine designed for cancer patients. Among other honours, Dr. Lupien is a recipient of the Mona Gauthier Award, the Canadian Cancer Society Bernard and Francine Dorval Award for Excellence, the Allan Slaight Collaborator Award, a three times recipient of the Investigator Award from the Ontario Institute for Cancer Research, a two times recipient of the Till and McCulloch Discovery of the Year award and co-founder of the CoBE ecosystem. Dr. Lupien earned his PhD in experimental medicine at McGill University under the leadership of Dr. Sylvie Mader and carried out postdoctoral training in medical oncology as an Era of Hope Fellow at the Dana-Farber Cancer Institute/Harvard Medical School under the mentorship of Dr. Myles Brown followed by a PLDA at Harvard Business School. Dr. Lupien joined the Princess Margaret Cancer Centre and the University of Toronto in 2012.

Tony Kouzarides

Senior group leader and Professor at the Gurdon Institute, University of Cambridge, and

Director and co-funder of the Milner Therapeutics Institute at the University of Cambridge, UK

Tony Kouzarides is Professor of Cancer Biology at the University of Cambridge. He is director and co-founder of the Milner Therapeutics Institute and a Senior Group leader at the Gurdon Institute. He did his PhD at the University of Cambridge and postdoctoral work at MRC Laboratory of Molecular Biology and New York University Medical Center. His research is focused on epigenetic modifications and their involvement in cancer. Tony is founder and director of Cambridge Gravity, a philanthropic vehicle for science at the University of Cambridge and a founder of a cancer charity "Vencer el Cancer" (Conquer Cancer) based in Spain. He is a co-founder and ex-director of Abcam plc, a publicly trading research reagents company in Cambridge, a co-founder and ex-director of Chroma Therapeutics, of a drug discovery company based in Oxford and a co-founder and current director of STORM Therapeutics, a drug discovery company based in Cambridge.

Direna Alonso Curbelo

Junior group leader at the Institute for Research in Biomedicine, Barcelona, Spain

Direna Alonso Curbelo graduated from Pharmacy School at the Complutense University of Madrid in 2007, and pursued her PhD studies in the laboratory of Dr. Maria Soengas, at the Spanish National Cancer Center (CNIO). Her graduate research focused on understanding howendolysosomal trafficking contributes to melanoma progression and drug response, and itsregulation by oncogenic and lineage-specific signaling (eg Cancer Cell 2014). After obtainingher PhD in 2013 with an Extraordinary Doctorate Award, she joined the laboratory of Dr ScottLowe at Memorial Sloan-Kettering Cancer Center (MSKCC), in New York City, as apostdoctoral fellow. During her postdoctoral training, she combined innovative mouse models,functional genomics tools and genome-wide profiling methods to understand how cell fatedysregulation, downstream of genetic and environmental insults, contributes to senescence andcancer development. As examples, her research uncovered epigenetic traits of senescent cellsthat promote their immune surveillance in pre-malignant and tumor settings (Cancer Discovery2016; BioRvix 2022). In parallel, applying new mouse models enabling spatiotemporally-controlled perturbation of epigenetic mechanisms in vivo, her work revealed that oncogenicKRAS and tissue damage cooperate to produce a cancer-associated chromatin state in pre-malignant pancreatic tissues that is required for tumor initiation and is retained throughoutmalignant progression (Nature, 2021).Throughout her scientific career, Direna has received several recognitions, including theBenjamin F. Trump Award for Scientific Research Excellence, the American Association ofCancer Research (AACR) Scholar-in-Training Award, or the prestigious Blavatnik RegionalAward for Young Scientists. Since January 2022, Direna is the principal investigator of the Inflammation, Tissue Plasticity &Cancer laboratory at IRB Barcelona. Focusing on pancreatic and liver cancers, the group studiesmolecular and microenvironmental mechanisms whereby genetic mutations and inflammationinteract to alter the normal identity of cells and their tissue niche to promote cancer, aiming touncover and exploit cancer-specific traits for its earlier detection and treatment.

Michaela Frye

Professor at the German Cancer Research Center (DKFZ), Heidelberg, Germany

Michaela Frye completed her PhD in Frankfurt/Main in Germany in 2000 studying the role of epithelial defensins in Cystic Fibrosis. In 2001, she joined Cancer Research UK (CR-UK) in London as a Postdoctoral Fellow, where studied how stem cells form and maintain adult tissues. In 2007, Michaela started her independent research group at the Wellcome Trust – Medical Research Council Cambridge Stem Cell Institute. She received a CR-UK Career Development Fellowship in 2007 and a CR-UK Senior Fellowship and an ERC Consolidator Grant in 2013 to study how dysregulation of stem cell function contributes to human diseases and cancer. In 2019, she accepted a Professorship at the DKFZ in Heidelberg Germany where her group studies mechanisms regulating gene expression regulate stem cell fate in normal tissues and cancer.

Kamil R Kranc

Professor at the Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, London, UK

Prof. Kamil R Kranc is a Chair of Haematology at Barts Cancer Institute in London. Kamil completed his MD degree at the Medical University of Silesia, Poland (1994-2000), a PhD in Biochemistry at the University of Oxford (2000-2003) and a postdoctoral training in immunology and stem cell biology also at Oxford (2003-2007). He was a Group Leader at the University of Oxford (2007-2010) and University of Glasgow (2010-2013). From 2013 to 2018, Kamil was a Professor of Molecular Haematology at the University of Edinburgh. He joined Barts Cancer Institute as a Professor/Chair of Haematology in 2018. The central aim of Kamil's laboratory is to understand the biology of leukaemic stem cells and identify therapeutic targets to specifically eradicate them. The lab focuses on targeting the epitranscriptome and hypoxia signalling pathways to achieve these goals.

Cristian Belodi

Group leader at the Lund Stem Cell Centre, Lund University, Lund, Sweden

Cristian Bellodi graduated cum laude from the University of Modena and Reggio Emilia, Italy, with a Bachelor of Science degree in Biological Sciences in 2002. As a graduate student, he joined Prof. Paolo Salomoni's group at the Medical Research Council (MRC) Toxicology Unit, Leicester, UK, and received his Ph.D. in Molecular and Cellular Biology from the University of Leicester in 2009. From 2009 to 2013, Dr. Bellodi trained as a postdoctoral fellow under the supervision of Prof. Davide Ruggero at the University of California, San Francisco (UCSF), USA. In 2014, Dr. Bellodi joined the Lund Stem Cell Center at Lund University to start his own research group. He currently holds an Associate Professor position within the Division of Molecular Hematology. The Bellodi's laboratory studies how RNA epigenetics and metabolism impact development and tumorigenesis by coupling mouse genetics with state-of-the-art sequencing approaches to unravel new gene expression facets at the interface between RNA modifications, splicing, and translation.

Marco Gerlinger

Professor of the Gastrointestinal Cancer Medicine and Group Leader at Cancer Research UK – Barts Centre, London, UK

Dr Marco Gerlinger studied Medicine in Munich, Wurzburg, London and Boston. After obtaining his MD from the Institute for Immunology in Munich, he undertook postdoctoral training in cancer immunotherapy at the University of Zurich. He specialized in Internal Medicine and Medical Oncology in Zurich and London. As a postdoc and subsequently as a clinician scientist at the Cancer Research UK London Research Institute, he investigated biomarkers of targeted cancer drug resistance and developed technologies to measure and track genetic and functional intra tumour heterogeneity and cancer evolution. Dr Gerlinger joined the ICR's Centre for Evolution and Cancer as a team leader in October 2013. He is also a Consultant Medical Oncologist at The Royal Marsden NHS Foundation Trust where he treats patients with gastrointestinal cancers. Dr Gerlinger joined CRUK-Barts Center in January of 2022 as a professor. Source: https://www.icr.ac.uk/our-research/researchers-and-teams/dr-marco-gerlinger

Christina Curtis

Professor at the Stanford University, School of Medicine, Departments of Medicine and Genetics, Stanford (CA), US

Christina Curtis, PhD, MSc is an Endowed Professor of Medicine and Genetics at Stanford University where she leads the Cancer Computational and Systems Biology group. Dr. Curtis also serves as the Director of Breast Cancer Translational Research and Co-Director of the Molecular Tumor Board at the Stanford Cancer Institute. Dr. Curtis's laboratory leverages computational modeling, high-throughput molecular profiling and experimentation to develop new ways to prevent, diagnose and treat cancer. Her research has helped to redefine the molecular map of breast cancer and led to new paradigms in understanding how human tumors evolve and metastasize. Dr. Curtis is the recipient of numerous awards, including those from the V Foundation for Cancer Research, STOP Cancer and the American Association for Cancer Research (AACR). She received the National Institutes of Health Director's Pioneer Award in 2018, the Stanford Prize in Population Genetics and Society (2020) and was named an In vivo Rising Leader in the Life Sciences (2021) and the Julius B. Kahn Visiting Professor in the Dept of Pharmacology, at Northwestern University (2020). In 2022 she received the AACR Award for Outstanding Achievement in Basic Science. Dr. Curtis is also Kavli Fellow of the National Academy of Sciences, a Susan G. Komen Scholar and a Chan Zuckerberg Biohub Investigator. Dr. Curtis serves as a scientific advisor to multiple academic institutes and biotech and was elected to the AACR Board of Directors in 2022. She also serves on the editorial board of journals spanning computational biology to precision oncology. Source: https://med.stanford.edu/curtislab.html

Vessela N. Kristensen

Director of Research and Head of Division of Research and Development at the Department of Medical Genetics and Professor at the Medical Faculty of the University in Oslo (UiO), Oslo, Norway

Vessela N. Kristensen is a Director of Research and Head of Division of Research and Development at the Department of Medical Genetics, OUS and Professor at the Medical Faculty of the University in Oslo (UiO). Previously she worked at the Department of Clinical Molecular Biology and Lab science (EpiGen), Akershus university hospital, and Group Leader at the Department of Genetics, IKF, Det Norske Radiumhospital. She has been also Professor II at the Centre for Integrative Genetics, University of Life Sciences, Ås and assistant professor at the Advanced Technology Center at NCI, NIH, Bethesda. Kristensen has worked in the Berzelius Laboratory at Karolinska to work on the functional characterization of polymorphic oxidative enzymes as well as at Fujita Health University, Nagoya, Japan. Kristensen's research interests are related to how genetic variation affects occurrence of somatic alterations, gene expression patterns and genome wide copy number alterations in human breast and ovarian tumors (http://www.ous-research.no/kristensen/). Understanding inherited genetic variability and how it affects crucial biological pathways is likely to lead to new successful prevention and treatment strategies. The tumor initiation, progression and clinical presentation are directly dependent on its genetic and biochemical environment – the entire body. This work has lead to the communication of 285 scientific papers since 2002. She is a recipient of several national and international grants and awards, member of the Norwegian Academy of Science and Letters, scientific and administrative boards in Norway and abroad and member of academic evaluating committees in Norway, Sweden, Denmark and Iceland. Current topics of research are in the field of genomic variation in relation to susceptibility, clinical presentation, treatment response and adverse side effects of treatment, gene regulation and proximal phenotypes (RNA expression and metabolic profiles) in breast cancer.

Chuan He

Professor at the Department of Chemistry and Department of Biochemistry and Molecular Biology at the University of Chicago, Chicago (IL), US

Dr. He is the John T. Wilson Distinguished Service Professor in the Department of Chemistry and Department of Biochemistry and Molecular Biology at the University of Chicago. He received his bachelor of science degree in 1994 from the University of Science and Technology of China and his Ph.D. in chemistry from the Massachusetts Institute of Technology in 2000, studying under professor Stephen J. Lippard. After training as a Damon-Runyon postdoctoral fellow with professor Gregory L. Verdine at Harvard University, he joined the University of Chicago as an assistant professor, rising to associate professor in 2008 and full professor in 2010. He was selected as an investigator of the Howard Hughes Medical Institute in 2013. Dr. He's research spans a broad range of fields including chemical biology, RNA biology, epigenetics, biochemistry, and genomics. His recent research concerns reversible RNA and DNA methylation in biological regulation. In 2011, his group discovered reversible RNA methylation as a new mechanism of gene expression regulation. His laboratory has spearheaded the development of enabling technologies to study the biology of RNA and DNA modifications.

Donate Weghorn

Group leader at the Centre for Genomic Regulation (CRG), Barcelona, Spain

Donate is a group leader at the Centre for Genomic Regulation (CRG) in Barcelona, Spain. She joined the CRG after spending her postdoc years at the Harvard Medical School in Boston, USA, where she focused on cancer genomics and statistical genetics. During her time as a PhD student at the Institute for Theoretical Physics in Cologne, Germany, Donate worked on biophysical and population genetics problems. The research of the group at the CRG focuses on the mathematical modeling and statistical analysis of DNA sequencing data from both cancer tumors as well as the human population. The two main aims that drive projects in the group are the probabilistic description of mutational processes and the inference of selection pressures, both in tumors as well as in the human germline genome.

Núria López-Bigas

ICREA professor at the Institute for Research in Biomedicine and Associate professor at the University PompeuFabra, Barcelona, Spain

She obtained her PhD in Biology at the University of Barcelona and has expertise in Medical Genetics and in Computational Biology and Bioinformatics. Next, she moved to the European Bioinformatics Institute in Hinxton (Cambridge, UK) to work on Computational Genomics and then at the Center for Regulatory Genomics (Barcelona). Núria joined the Pompeu Fabra University in 2006 with a Ramón y Cajal Position and was appointed ICREA Research Professor in October 2011. Her lab moved to IRB Barcelona in 2016. Among other awards, she received the ERC Consolidator Grant in 2015, the XI Banco Sabadell Foundation Award for Biomedical Research in 2016, and the IX Premio Nacional de Investigación en Cáncer "Doctores Diz Pintado" in 2019.

Zohar Yakhini

Head of Machine Learning and Data Science Program and Proffesor at EfiArazi School of Computer Science, Herzliya, Israel

Prof' Yakhini's research focuses on the applications of statistics and data science in molecular biology. He pioneered the bioinformatics practice at Agilent Laboratories in 1997 and in following years recruited, trained and worked with top scientists and students at Agilent and at the Technion, leading to significant commercial and scientific achievements. Prof' Yakhini is a faculty member in computer science at the Interdisciplinary Center in Herzeliya and visiting faculty member at the computer science department at the Technion in Haifa. He leads an active bioinformatics research group with students in both institutes. He has been Master Scientist at Agilent Laboratories until April 2016 and is currently active in industry consulting and SAB roles. At Agilent Prof' Yakhini developed data analysis methods for Agilent's early generation microarrays and later for Agilent's commercially successful array CGH technology. His data analysis methods work, focused on molecular measurement, led to more Agilent products, as well as to significant scientific studies and publications. In recent years Prof' Yakhini worked on design and data analysis to enable the efficient and effective use of synthetic DNA and on other aspects of synthetic biology. Work from Prof' Yakhini's academic research group includes the development of statistical methods to analyze ranked lists as employed, for example, in the popular analysis tool GOrilla. The group also participated in several successful collaborations, applying advanced data analysis approaches in the context of advanced molecular biology studies. Prof' Yakhini holds a PhD degree in Mathematics from Stanford University and a BSc in Computer Science from the Hebrew University in Jerusalem. He is the inventor of 12 issued US patents and has a publication h-index of 40. Source: https://www.runi.ac.il/en/faculty/zyakhini

José Antonio Seoane

Head of Cancer Computational Biology group at the Vall d'Hebron Institute of Oncology, Barcelona, Spain

José A. Seoane is the head of Cancer Computational Biology group at the Vall d'Hebron Insitute of Oncology (Barcelona, Spain). He completed his PhD in Computer Science at the University of A Coruña (Spain) in 2012. After that, he conducted his first postdoc at the School of Social and Community Medicine at the University of Bristol (UK) under supervision of Tom Gaunt and Colin Campbell and then he moved to US, first to the University of Southern California and later to Stanford University to the group of Christina Curtis. Dr Seoane main interests are how different layers of (epi)genomic data can be integrated in order to establish a holistic view of the molecular mechanism underlying cancer initiation, progression, drug resistance and metastasis.

Óscar Rueda

Group leader at the MRC Biostatistics Unit at the University of Cambridge, Cambridge, UK

Óscar Rueda has a PhD in Mathematics (Statistics) from the University of Valladolid, Spain. He has previously conducted research at the Spanish National Cancer Centre (CNIO) as a graduate student working on Bayesian methods for detecting copy number aberrations in DNA and at CRUK (University of Cambridge) as a postdoc, in the Caldas Lab, working on METABRIC and in many other projects analysing breast cancer data from tumours and pre-clinical models. Since September of 2020 he leads a group at the MRC Biostatistics Unit (University of Cambridge) devoted to the development of statistical models for the analysis of large genomic and transcriptomic datasets in order to identify biomarkers that can be used to stratify patients and to identify potential drug candidates for specific breast cancer subtypes.

Salvatore Fabbiano

Scientific Editor of Med journal (Cell group), Madrid, Spain

Salvatore Fabbiano received his Ph.D. in Physiology at the University of Salamanca, Spain, where he studied signaling pathways involved in the development of cardiometabolic diseases in the lab of Xosé R. Bustelo. He then conducted his postdoctoral research in the lab of Mirko Trajkovski at the University of Geneva, Switzerland, focusing on immunometabolism and host-microbiota homeostasis in metabolic disease. Driven by a broad interest in scientific research, he joined Cell Metabolism as scientific editor in July 2018 and moved to Med, Cell Press' new flagship medical title, in November 2019.

Ilse Ariadna Valtierra Gutierrez

Senior Editor of Nature Communications, Berlin, Germany

Ilse is an Associate Editor at Nature Communications. She joined the Cancer team of the journal in June 2020 and handles manuscripts in different areas of cancer genomics, heterogeneity and evolution. She has a PhD from the Ludwig Maximilian University of Munich, where she studied clonal heterogeneity and evolution in acute myeloid leukaemia using genomics and single-cell transcriptomics. Before that, she obtained her MSc in Molecular Biosciences from Heidelberg University, and her BSc in Genomic Sciences from the National Autonomous University of Mexico. She is based in Berlin.

Javier Carmona

International scientific strategy and institutional partnerships of the Valld'Hebron Institute of Oncology (VHIO), Barcelona, Spain

Javier Carmona studied Biology between the University of Navarra and the Autonomous University of Madrid. During his graduate studies in the laboratory of Dr Manel Esteller, he studied alterations in DNA methylation in cancer metastasis. After obtaining his PhD in 2013 with Extraordinary Doctorate Award, he joined the lab of Dr. José Baselga as a postdoc fellow at Memorial Sloan-Kettering Cancer Center in New York City, USA, where he investigated mechanisms of resistance to therapy in patients with HER2-driven breast cancer. In 2016, Javier joined Nature Medicine as scientific editor handling manuscripts in translational cancer research, clinical oncology and artificial intelligence in biomedicine, and in 2019 he became Deputy Editor at the journal. During this time, he also collaborated as freelance editor for other journals in the Nature portfolio, such as Nature Communications, Nature Cell Biology and npj Precision Oncology. Since February 2022, he works at Valld'Hebron Institute of Oncology (VHIO) in Barcelona, Spain, where he supports the international scientific strategy and institutional partnerships of the center, and keeps actively involved in scientific communication and publishing.

SPEAKERS' ABSTRACTS

1. Epigenomics and cancer

S-1

Cancer epigenomics and epitranscriptomics: from knowledge to applications

Manel Esteller

Josep Carreras Leukaemia Research Institute, Spain

For the last twenty-five years an increasing amount of evidence has shown the relevance of epigenetics in cell biology and tissue physiology, being DNA methylation aberrations in cancer the flag-ship for the recognition of its disturbance in human diseases. From the candidate gene approaches, new powerful technologies such as comprehensive DNA methylation microarrays and whole genome bisulfite sequencing has recently emerged that have reinforced the notion of epigenetic disruption in the crossroad of many sickness. From the poster-boy cases of MGMT and GSTP1 hypermethylation in the prediction of alkylating drug response and prostate cancer detection, respectively, to the personalized treatment of leukemia with small molecules targeted to fusion proteins involving histone modifiers, the field has walked a long path. The current talk will focus in the epigenetic profiling, basically at the level of DNA methylation and histone modifications, that is starting to provide clinical value in the diagnosis, prognosis and prediction of response to drug therapies. For cancer, we have already a wide view of the undergoing DNA methylation events that expand beyond classical promoter CpG islands of tumor suppressor genes and we have a growing list of mutated chromatin remodeler genes that contributes to the tumorigenesis process. It is time to apply this knowledge in practical clinical situations like the diagnosis of cancers of unknown primary, the screening of malignancies in high-risk populations or a biomarker selection of the patients that should receive treatment with anticancer drugs. Beyond our comfort zone, we should be aware that chemical modifications not only affect the DNA molecule, but also RNA. The epigenetics of RNA or the analysis of the epitranscriptome represents another relevant step to understand the complex relationship between genotypes and phenotypes in human tumors.

S-2

Chromatin variants in cancer

Mathieu Lupien

University of Toronto, Canada
Princess Margaret Cancer Centre, University Health Network, Toronto, Canada.

Precision oncology relies on finding the genetic drivers of cellular transformation (aka: cancer drivers) within coding and non-coding DNA elements of the cancer genome, pairing cancer drivers to drug sensitivity, and designing clinic "ready" companion tests to help guide treatment selection. The gold standard approach employed in the clinic relies on using genetic variants to find cancer driver DNA elements. However, focusing on genetic variants alone yields actionable targets for fewer than 30% of cancer patients, raising the need for alternatives approaches to find cancer drivers. Chromatin variants are a form of genomic variation fundamental to normal cell fate determination, which inactivate or activate coding and non-coding DNA elements throughout cellular differentiation. Despite chromatin variation being a well-established feature of genetic drivers to normal development, identifying and understanding the role of chromatin variants in cancer is needed for a new era in precision oncology using all forms of genomic variation to find cancer drivers. Here, we present our latest methodologies to identify genetic drivers of oncogenesis from chromatin variants and present evidence for using chromatin variants in the development of alternative therapeutic approaches against cancer, a needed step towards delivering on the promise of precision oncology.

S-3

Targeting RNA modifying enzymes in the treatment of cancer.

Tony Kouzarydes

Gurdon Institute and Milner Therapeutics Institute, University of Cambridge, UK

Our lab is interested in identifying RNA modifying enzymes involved in cancer in order to target these enzymes for drug discovery. We have identified the METTL3 enzyme, that modifies m6A, as a regulator of AML-leukaemia via a chromatin-based pathway. This mechanism involves METTL3 being targeted to the promoter of leukaemia genes, via a specific transcription factor, to regulates their expression. A small molecule has been developed that inhibits the catalytic activity of METTL3. This molecule is highly specific for METTL3 and can phenocopy the cellular consequences of a METT3 knock-down, in cells and models systems.

The METTL3 inhibitor will be in human clinical trials this year. This is the first drug in this class to enter clinical trials, demonstrating that RNA modifying enzymes are druggable, and that other RNA modifying enzymes may be targets for drug discovery. We are now investigating other potential enzyme targets to be targeted against cancer. One of these, which we can show is implicated in pancreatic cancer, in cell and mouse models, will be presented.

S-4

Epigenetic licensing of epithelial-immune interactions in cancer.

Direna Alonso Curbelo

Institute for Research in Biomedicine, Spain

Cancer results from a complex interaction between genetic mutations and environmental insults that triggers changes in cell identity and tissue state. These changes are highly reminiscent of wound healing processes yet, paradoxically, contribute to cancer development and metastatic progression. To understand how physiological plasticity goes awry during tumor development, we combined single-cell profiling methods and functional genomics tools to characterize and perturb molecular and cellular networks defining normal, inflamed, pre-malignant and malignant tissues in autochthonous models of pancreatic cancer. We uncover aberrant chromatin states in the pancreatic epithelium uniquely induced by the cooperative action of tissue damage and cancer-predisposing mutations (oncogenic KRAS), that distinguish neoplastic transformation from normal regeneration and which are selected for during tumor evolution. These early epigenomic alterations endow discrete epithelial cell populations with an enhanced capacity to interact with the immune cells recruited to inflamed environments and establish feedback communication loops that define and direct tumorigenesis. We propose that a better understanding of the epigenetic mechanisms mediating interplay between genetic mutations, environmental cues and cell identity programs will expose specific vulnerabilities of incipient and advanced tumor cells late that may be exploited for cancer interception and treatment.

2. Cancer epitranscriptomics

S-5

Targeting RNA modifications in cancer.

Michaela Frye
DKFZ, Germany

Many of the hundreds of known chemical modifications in RNA were discovered over forty years ago but then forgotten because suitable, sensitive tools to detect the modifications at high resolution were lacking. Through the development of novel biochemical, functional and genomics tools we are only now beginning to understand the whole breadth and extensive functional roles of RNA modifications in higher organisms. I will present some mechanistic examples how RNA modifications help to shape normal tissue homeostasis, and how aberrant formation of RNA modifications contributes to disease such as cancer. By focusing on the roles of RNA methylation, I will discuss novel and emerging molecular functions of RNA modifications in regulating mRNA translation. Together, our work demonstrates that by understanding the role of RNA modifications in physiology and pathology, novel and powerful therapeutic drug targets for human diseases and can potentially be identified and further optimized for clinical studies.

S-6

Targeting mRNA modifications to eradicate cancer stem cells in acute myeloid leukaemia.

Kamil R Kranc
Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, UK

Haematopoiesis critically depends on haematopoietic stem cells (HSCs), which possess unique self-renewal capacity and multilineage differentiation potential, replenishing all blood cells. Acute myeloid leukaemia (AML) is an aggressive disorder of HSCs and progenitors, which acquire driver mutations to generate treatment-resistant leukaemic stem cells (LSCs). LSCs fuel the over-proliferation of primitive myeloid progenitors (blasts), which damage the bone marrow and multiple organs, resulting in a widespread tissue devastation. Current AML therapies are toxic to normal haematopoiesis, but often fail to eliminate LSCs. The surviving population of LSCs drives minimal residual disease, ultimately causing fatal disease relapses. Given the sobering survival outcomes, it is critical to identify new therapeutic targets for selective LSC elimination and design improved non-toxic treatments. This challenge encouraged many laboratories to turn to new fields in search of novel therapeutic targets. One such field is the epitranscriptome, encompassing chemical RNA modifications, whose immense functional significance in physiology and cancer is beginning to emerge.

The mRNA N6-methyladenosine (m6A) modification is an emerging regulator of normal and malignant haematopoiesis. m6A is installed by writers, removed by erasers, and its functions are executed by m6A readers, including the YTHDF proteins. During my presentation, I will discuss our most recent findings indicating the roles of YTHDFs in normal and malignant haematopoiesis.

3' terminal uridylation of mRNA's poly(A) tail is a key determinant of mRNA turnover. The shortening of the poly(A) tail results in the recruitment of the terminal uridylyl transferases 4 and 7 (TUT4 and TUT7, respectively), which catalyse 3' uridylation of mRNA. During my talk, I will present our most recent data demonstrating the key significance of TUT4/7 in HSC biology and leukaemic transformation.

S-7

m6A-driven translation circuitry steers splicing directing genome integrity and leukemogenesis.

Cristian Bellodi

Lund Stem Cell Centre, Lund University, Sweden

SF3B1 is the most mutated splicing factor in myelodysplastic syndromes (MDS), clonal hematopoietic disorders with variable risk of leukemic transformation. Although tumorigenic SF3B1 mutations have been extensively characterized, the role of "non-mutated" SF3B1 in cancer remains largely unresolved. Here we identify an evolutionarily conserved N6- methyladenosine (m6A) epitranscriptomic program that steers SF3B1 levels to counteract leukemogenesis. Our analysis of human and murine pre-leukemic MDS cells reveals dynamic regulation of SF3B1 protein abundance, which impacts MDS-to-leukemia progression in vivo.

Mechanistically, we uncover that ALKBH5-mediated 5'UTR m6A demethylation fine-tunes SF3B1 protein levels by directing start site selection during translation initiation. Furthermore, we find that m6A-driven modulation of SF3B1 translation critically coordinates the splicing of central DNA repair and epigenetic regulators during transformation. This translation-splicing regulatory circuit impacts genome stability and leukemia progression in vivo, supporting integrative analysis in humans that SF3B1 molecular signatures may predict mutational variability and poor prognosis. These findings highlight a post-transcriptional gene expression nexus that unveils unanticipated SF3B1-dependent cancer vulnerabilities.

S-8

Gene expression regulation through RNA methylation.

Chuan He

Department of Chemistry, Department of Biochemistry and Molecular Biology, Institute for Biophysical Dynamics, Howard Hughes Medical Institute, The University of Chicago, USA

Over 170 types of post-transcriptional RNA modifications have been identified in all kingdoms of life. We have previously discovered two RNA demethylases, FTO and ALKBH5, which catalyze oxidative demethylation of the most prevalent modifications of mammalian messenger RNA (mRNA) and other nuclear RNA, N^6-methyladenosine (m^6A). We have recently discovered m^6A methylation of chromatin-associated regulatory RNAs (carRNAs), which modulates chromatin state and transcription in mammals. These findings suggest that reversible RNA modification could impact biological regulation analogous to the well-known reversible DNA and histone chemical modifications. I will present recent mechanistic investigation of chromatin and transcription regulation through m6A methylation and demethylation, and impacts on mammalian development as well as cancer progression.

3. Cancer genomics, tumor evolution and heterogeneity

S-9

Signature decomposition of tumor mutation spectra using artificial neural networks.

Donate Weghorn

Centre for Genomic Regulation, Spain

Seen from an evolutionary perspective, cancer is a complex system subject to high mutation rates and strong selection pressures. Mutations, the substrate of selection, are caused by many different mutational processes. A multitude of such mutational processes, or "signatures", has been identified and associated with biochemical mechanisms of DNA lesions and repair. The mutational spectrum of any given tumor can be decomposed into these signatures in order to classify tumors into subtypes, determine exposure times to certain mutagens or characterize individual mutation origins. State-of-the-art methods to quantify the contributions of different mutational processes to a tumor sample fail to detect certain mutational signatures, only work well for a relatively high number of mutations, do not provide error estimates of signature contributions, and do not readily extend to the newest signature catalogs. Here, I will describe SigNet, a novel approach to signature decomposition based on an artificial neural network. By leveraging the correlations between signatures present in real data, this approach outperforms existing methods, particularly for samples with few mutations. We applied SigNet to elucidate the effects of hypoxia on the tumor mutational footprint and discovered novel and known correlations of a set of mutational signatures with hypoxia, including a strong association of hypoxia with a decrease in the activity of DNA repair processes.

4. Integrated omics and machine learning in cancer

S-10

Tumor genomes shed light into somatic mutational processes and cancer vulnerabilities.

Núria López-Bigas

Institute for Research in Biomedicine and University Pompeu Fabra, Spain.

Somatic mutations are the driving force of cancer genome evolution. The rate of somatic mutations appears to be greatly variable across the genome due to variations in chromatin organization, DNA accessibility and replication timing. In addition, other variables that influence the mutation rate in a local scale are starting to emerge. I will discuss recent findings from our lab on how genome conformation influences mutation rate. These findings have important implications for our understanding of mutational and DNA repair processes, genome evolution and in the identification of cancer driver mutations.

Given the evolutionary principles of cancer, one effective way to identify cancer genes is by tracing the signals left by the positive selection of driver mutations across tumours. Using this concept we analyze thousands of tumor genomes to generate a compendium of cancer genes across tumor types (http://www.intogen.org), and we build machine learning models inspired in evolutionary biology that effectively identify driver mutations in each gene and cancer type (http://www.intogen.org/boostdm). The results (integrated in CancerGenomeInterpreter.org) contribute to the interpretation of tumor mutations in precision cancer medicine.

Somatic mutations may also drive clonal expansions in normal tissues, such as clonal hematopoiesis. We have performed a "reverse calling" to reliably identify blood somatic mutations in 12000 cancer patients. Repurposing methods of cancer genomics we identify genes and mutations driving clonal hematopoiesis (http://www.intogen.org/ch).

S-11

Spatial biology - how data analysis can push the envelope.

Zohar Yakhini

EfiArazi School of Computer Science, Reichman University, Israel.

Spatial relationships, in the context of cells, cell components and cell types have been studied in biology and medical science for many decades. In recent years, however, there has been a great progress in molecular biology measurements, at the single cell and spatial resolution. We will describe work on several data analysis aspects of spatial and single cell biology. In particular, we will describe algorithmics and results on inferring gene expression from H&E as well as the use of statistical enrichment methods to infer miRNA activity in spatial transcriptomics samples.

S -12

Deciphering subtype specific epigenetic regulators in breast cancer.

José Seoane

Vall d'Hebron Institute of Oncology, Spain.

Epigenetic dysregulation is a key factor in the development of tumor malignancies, but also determining the outcome of the patient and the resistance to specific drugs. In breast cancer subtypes, methylation-based regulation has been described previously, but the different accessibility landscape across subgroups has not been studied yet. In this work we propose a method to discover subtype specific epigenetic regulation and discuss the finding in breast cancer. We investigate the specific role of a chromatin regulatory gene in the accessibility of basal subtype, which also acts as an epigenetic regulator in luminal subtype trough methylation and is associated with outcome. Finally, we investigate how hormone treatment changes the accessibility landscape.

S-13

Machine learning for prognosis and prediction in breast cancer.

Óscar Rueda

MRC Biostatistics Unit at the University of Cambridge, UK.

The increasing availability of complex data types available in biomedical studies require a balanced data analysis approach that maintains the good properties of classical statistical methods and the predictive ability of modern machine leaning techniques. We describe here two examples, the development of a predictor of response to Neo adjuvant therapy in breast cancer and a model to monitor progression in metastatic breast cancer, that uses principles from both disciplines.

SHORT TALKS' ABSTRACTS

ST-1

Reshaping the role of METTL3 in breast tumorigenesis.

Francesca Aguilo, Cyrinne Achour, Devi Prasad Bhattarai, Margalida Esteva.

Wallenberg Center for Molecular Medicine (WCMM), Department of Molecular Biology, Umea University, Sweden.

METTL3 is the solely catalytic subunit of the m6A-methylase complex regulating most aspects of the mRNA life cycle including pre-mRNA processing, nuclear export, decay and translation. m6A deposition occurs co-transcriptionally in the nucleus. However, cytoplasmic localization of METTL3 has also been shown for some types of cancer. Here we show that METTL3 is highly expressed in the cytoplasmic compartment of breast cancer cells from patients. We found that the cytoplasmic METTL3 interacts with the exocyst complex, an evolutionary conserved octameric complex of proteins that mediates intracellular membrane trafficking i.e., the targeting and tethering of post-Golgi secretory vesicles to specific membrane sites. Noteworthy, breast cancer cell lines depleted of METTL3 displayed less gelatinases activity and less invadopodia formation, supporting the role of METTL3 in cell invasion via exocytosis. Altogether, our findings revealed a novel non-canonical role for METTL3 in the cytoplasm and its adverse consequences for breast cancer growth and progression.

ST-2

Deciphering the role of cell phenotypes in response to treatment using explainable Machine Learning.

Youness Azimzade, Olav Engebraaten , Arnoldo Frigessi , Alvaro Köhn-Luque and Vessela Kristensen.

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Tumors are heterogeneous and they contain a large set of cell types. The relevant and not somewhat addressed question is how different cell phenotypes contribute to treatment response.

We study this question in the context of neoadjuvant chemotherapy of breast cancer. We first find the frequency of phenotypes from bulk GEPs using deconvolution. Then we develop a Machine Learning model to predict response to neoadjuvant based on fractions of different cell types and other pathological features. We run a post-hoc explanation on our ML model to find the role of cell phenotypes and pathological features in treatment response. Based on the knowledge we gain from this analysis, we define a predictive score combining the most essential cell types than can separate responders and nonresponders. We validate this score on external datasets. We study this question in the context of neoadjuvant chemotherapy of breast cancer. We first find the frequency of phenotypes from bulk GEPs using deconvolution. Then we develop a Machine Learning model to predict response to neoadjuvant based on fractions of different cell types and other pathological features. We run a post-hoc explanation on our ML model to find the role of cell phenotypes and pathological features in treatment response. Based on the knowledge we gain from this analysis, we define a predictive score combining most essential cell types than can separate responders and nonresponders. We validate this score on external datasets.Tumors are heterogeneous and they contain a large set of cell types. The relevant and not fairely addressed question is how different cell phenotypes contribute to treatment response.

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ST-3

Study of the genomic landscape of lung and bladder tumors to find potential biomarkers in immunotherapy response.

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Immune checkpoint inhibitors (ICIs) have become one of the most promising therapeutic approaches for the oncological patients care, as they have allowed the achievement of long survivals in different tumor types. Nevertheless, the rate of responder patients is low yet and, hence, the discovery of predictive response biomarkers is crucial. Genomic instability and high diversity of tumor neoantigens have been associated to ICIs response and their role in modulating tumor immunity has emerged recently. Here, we aim to obtain a genomic signature predictive of tumor response to ICIs by (a) evaluating Tumor Mutation Burden (TMB) as predictive biomarker, (b) analyzing specific mutations potentially related to ICIs response/resistance, (c) estimating Mobile Element Insertions (MEIs) as neoantigen sources, and (d) studying HLA haplotypes and T-Cell Receptor (TCR) repertoire populations to understand the tumor microenvironment (TME) role as potential predictive biomarker.

To achieve our objectives, FFPE tumor samples of 15 advanced lung cancer (LC) and 10 bladder cancer (BC) patients treated with first-line immunotherapy were analyzed by Whole Genome Sequencing (WGS). First, we performed a variant calling analysis using Mutect2 to calculate TMB and identify exclusive mutations in responder (R) and non responder (NR) patients, each group representing approximately half of them. Second, we employed TraFiC and ERVCaller to analyze the landscape of MEIs of these patients, LiLAC to haplotype their HLA genes and MiXCR to study their TCR repertoire.

We identified a higher TMB among R patients in BC, as well as exclusive SNVs and exclusively mutated genes in R and NR both in LC and BC. Interestingly, we detected a particular SNV in KRAS (p.Gly12Cys) exclusively in LC R patients. Specific mutational signatures were identified according the response. Moreover, particular HLA haplotypes, such as A*02:01, were more frequently found in R patients for both LC and BC. Finally, HLA LOH was more frequent among R patients with LC.

In conclusion, our comprehensive study represents a promising strategy for precision oncology, as our predictive genomic candidate biomarkers can favor the patient's selection more likely to benefit from immunotherapy, both in LC and BC.

ST-4

An integrated 'omics approach highlights the role of epigenetic events to explain and predict response to neoadjuvant chemotherapy and bevacizumab.

Thomas Fleischer, Mads Haugland Haugen, Jorgen Ankill, Laxmi Silwal-Pandit, Anne-Lise Borresen-Dale, Ingrid Hedenfalk, Thomas Hatschek, Jörg Tost, Olav Engebraaten, Vessela N. Kristensen.

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Through integration of DNA methylation and gene expression data from breast cancer tumors, we have shown that important cancer-driven pathways are potentially under epigenetic control through loss of enhancer methylation facilitated by chromatin loops [1, 2].

In our current work we have leveraged information from these cancer-associated methylated sites to predict response to chemotherapy combined with the anti-angiogenic drug bevacizumab. Here, we have 'omics data from fresh frozen tumor biopsies taken before, during and after treatment, and we report that DNA methylation at enhancer CpGs related to cell cycle regulation can predict response to the treatment with high fidelity (AUC=0.874), and we validate this observation in an independent patient cohort with similar treatment regimen (AUC=0.762). We also show that combining the DNA methylation score with a previously reported proteomic score, the prediction accuracy further improved in the validation cohort (AUC=0.784).

To study the functional mechanisms leading to resistance or response, we performed an integrative analysis on alterations in DNA methylation and gene expression levels, and we show that the epigenetic alterations that occur during treatment are different between responders and non-responders and that these differences may be explained by the proliferation-EMT axis through the activity of the transcription factor GRHL2. Taken together, these results illustrate the clinical benefit of the addition of bevacizumab to chemotherapy if administered to the correct patients.

1. Fleischer, T., et al., DNA methylation at enhancers identifies distinct breast cancer lineages. Nat Commun, 2017. 8 (1): p. 1379.

2. Ankill, J., et al., Epigenetic alterations at distal enhancers are linked to proliferation in human breast cancer. NAR Cancer, 2022. 4 (1): p. zcac008.

ST-5

Epigenetics meets invasion: an insulator element orchestrates a nine matrix-metalloproteinase cluster expression in triple-negative breast cancer.

Pere Llinàs-Arias (1), Javier J. I. Orozco (2), Miquel Ensenyat-Mendez (1), Sandra Íñiguez-Muñoz (1), Betsy Valdez (2), Anja Mezger (3), Borja Sesé (1), Eunkyoung Choi (3), Yan Zhou Tran4, Liqun Yao (4), Franziska Bonath (5), Chuan Wang (4), Mattias Ormestad (3), Alexander Boiko6, Manel Esteller (7-10), Maggie L. DiNome (11), Diego M. Marzese (1).

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Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype that displays high rates of distant metastases and lacks targeted therapies. The intrinsic mechanisms involved in metastatic spread/dissemination are not fully explored. In this regard, we aimed to unveil the epigenetic regulation of a gene regulatory network involved in invasion, the first step of the metastatic process. We focused on the matrix metalloproteinase (MMP) genes since this enzyme family is involved in basal layer degradation. We identified a particular genomic locus (chr11q22.2) where nine different MMPs are encoded. Data from genomic location, RNA expression levels, chromatin accessibility, and chromatin conformation led us to identify that this locus may be regulated by a master insulator element (IE) located near the MMP8 gene, leading to a topologically associating domain (TAD) insulation of nine MMP genes. Importantly, concomitant expression of the insulated MMP genes (MMP1, 3, and 10) is associated with significantly shorter relapse-free survival (log-rank P = 0.005, HR = 2.65 [1.31 – 5.37]) in TNBC patients. We, therefore, disrupted the CCCTC-Binding Factor (CTCF) binding site at the MMP8 IE with CRISPR/Cas9 technology in two TNBC cellular models. Then, we combined ATAC-seq and RNA-seq with functional experiments to determine the biological consequences of the IE impairment. Downregulation of the protumorigenic MMP1, 3, and 10 genes and upregulation of the antitumorigenic MMP8 gene were observed upon IE disruption. These findings highlight the relevance of regional regulatory mechanisms in TNBC during the metastatic cascade. Noteworthy, chromatin conformational changes impact TNBC cell phenotype and survival of TNBC patients.

ST-6

Somatic RNA-seq single nucleotide and indels variants discovery pipeline.

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Mutations accumulate over time and generate clonal and subclonal populations that causes intratumoral heterogeneity (ITH). Many bioinformatics pipelines have been developed to calculate ITH from DNA bulk sequencing data. The input of these methods are somatic variants found in the tumour sample; hence attaining accuracy and reproducibility in their calling are of outmost importance. However, not all somatic events that are detected in DNA sequencing data have a functional effect. We hypothesize that integrating RNA sequencing data to the analysis will broaden the landscape of ITH.

Best practices to identify variants from DNA sequencing data are already established such as the Genome Analysis Tool Kit (GATK) but the same is not applied for RNA. Somatic RNA variant calling is more challenging due to specific features such as coverage fluctuations, RNA editing, high number of duplicated reads and alternative splicing among others.

To profile ITH using DNA and RNA variants, we have developed a method to call mutations using both sequencing data. Moreover, RNA variant calling best practices are presented in the pipeline to supply the lack of standardisation in the community and to provide a reproducible and scalable resource to use. A comparison with previous work from Yizhak et al., 2019 (Science) was performed to demonstrate the robustness of the method. The analysis of The Cancer Genome Atlas (TCGA) cohort of samples used in previous pipeline has allowed us to compare the two methods successfully. Novel features of this method include the display of mutational patterns with indels and multiple nucleotide polymorphisms and RNA-specific signatures such as RNA editing.

ST-7

Cis-regulatory mutations associate with transcriptional and post-transcriptional deregulation of the gene regulatory program in cancers.

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Most cancer alterations occur in the noncoding portion of the human genome, where regulatory regions control gene expression. The discovery of noncoding events altering the cells' regulatory program has been limited to few examples with high recurrence or high functional impact. In this study, we show that transcription factor binding sites (TFBSs) have similar mutation loads to those in protein-coding exons. By combining cancer somatic mutations in TFBSs and expression data for protein-coding and miRNA genes, we evaluate the combined effects of transcriptional and post-transcriptional alterations on the regulatory program in cancers. The analysis of seven TCGA cohorts culminates with the identification of protein-coding and miRNA genes linked to mutations at TFBSs that are associated with a cascading trans-effect deregulation on the cells' regulatory program. Our analyses of cis-regulatory mutations associated with miRNAs recurrently predict 12 mature miRNAs (derived from 7 precursors) associated with the deregulation of their target gene networks. The predictions are enriched for cancer-associated protein-coding and miRNA genes and highlight cis-regulatory mutations associated with the dysregulation of key pathways associated with carcinogenesis. By combining transcriptional and post-transcriptional regulation of gene expression, our method predicts cis-regulatory mutations related to the dysregulation of key gene regulatory networks in cancer patients.

ST-8

m6A RNA modifications in the development and treatment of intestinal inflammation and malignancies mediated by XPO1.

Ane Olazagoitia-Garmendia (1), Henar Rojas-Marquez (1), Linda Zhang (2), Maria Del Mar Romero (3), Paula Mera (3), Laura Herrero (3), Dolors Serra (3), Cheng Luo (4), Chuan He (2) and Ainara Castellanos-Rubio (5).

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N6-methyladenosine (m^6A) RNA modifications regulate RNA metabolism in diverse ways and have been implicated in the development of different diseases, including intestinal pathologies. It is known that m^6A levels change in response to different cellular stress signals to activate various biological pathways and influence gene expression. Moreover, the importance of m^6A to the etiology of complex traits was recently highlighted.

XPO1 protein harbors an inflammation associated SNP in its 5'UTR, closely located to 3 m^6A motifs. We found that the 5'UTR of XPO1 mRNA is allele-specifically methylated in intestinal epithelial cells and that YTHDF1 reader protein mediates XPO1 translation. We observed that differential methylation levels influence XPO1 protein amounts contributing to the intestinal inflammatory environment.

We also described the effect of gluten (the triggering agent of celiac disease and related to increased risk to develop colon cancer) to induce m^6A levels, together with YTHDF1 and XPO1 protein levels and a subsequent NFkB activation. Moreover, YTHDF1, XPO1 and CXCL8 pro-inflammatory cytokine (regulated by NFkB) are upregulated in celiac disease as well as in several carcinomas (i.e. colon adenocarcinoma, esophageal carcinoma and stomach adenocarcinoma). Interestingly, when using two YTHDF1 specific inhibitors (compounds from traditional medicines) we were able to revert the gluten-induced increase of XPO1 protein and to ameliorate downstream inflammation in vitro and in vivo.

Our results point that m^6A methylation is involved in the development of intestinal pathologies by an increase in XPO1 protein levels that lead to inflammation. We found that both genetic predisposition and environmental factors (i.e. dietary gluten ingestion) contribute to m6A mediated XPO1 induction. Moreover, we confirmed that inhibition of YTHDF1, using two different compounds, can counteract the induction of XPO1 avoiding the activation of NFkB. To sum up, we have described a novel m6A regulated pathway implicated in intestinal disease development and propose novel therapeutic options for thesedisorders.

ST-9

Multivariate analysis of RNA chemistry marks uncovers epitranscriptomics-based biomarker signature for glioma diagnostics.

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One of the main challenge in cancer management relates to the discovery of reliable biomarkers, which could guide decision-making and predict treatment outcome. In particular, the rise and democratization of high-throughput molecular profiling technologies bolstered the discovery of "biomarker signatures" that could maximize the prediction performance. Such approach was largely employed from diverse OMICs data (i.e. genomics, transcriptomics, proteomics, metabolomics) but not from epitranscriptomics, which encompasses more than hundred biochemical modifications driving the post-transcriptional fate of RNA: stability, splicing, storage, and translation. Others and we have studied chemical marks in isolation and associated them with cancer evolution, adaptation, as well as response to conventional therapy. In this study, we have designed a unique pipeline combining multiplex analysis of epitranscriptomic landscape by high-performance liquid chromatography-coupled to tandem mass spectrometry with statistical multivariate analysis and machine learning approaches in order to identify biomarker signatures that could guide precision medicine and improve disease diagnosis. We applied this approach to analyze a cohort of glioma patients and demonstrate the existence of an "epitranscriptomics-based signature" that permits to discriminate and predict glioma grades with unmet accuracy. This study demonstrates that epitranscriptomics (co-)evolves along cancer progression and opens new prospects in the field of omics molecular profiling and personalized medicine.

Reference: Relier et al.

Multivariate Analysis of RNA Chemistry Marks Uncovers Epitranscriptomics-Based Biomarker Signature for Adult Diffuse Glioma Diagnostics.

Analytical Chemistry. 6;94(35):11967-11972. doi: 10.1021/acs.analchem.2c01526. 2022.

POSTERS' ABSTRACTS

P-1

MiR-182-5p and miR-375-3p in blood plasma as prostate cancer biomarkers.

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Prostate cancer (PCa) represents a malignancy with the highest prevalence and very high incidence among men worldwide. Clinical challenge is to differentiate localized PCa from benign prostate hyperplasia (BPH) due to the lack of specificity of routinely used biomarker PSA. Epigenetic biomarkers in liquid biopsies, especially miRNA, could address this challenge. The absolute expression of miR-375-3p, miR-182-5p, miR-21-5p, and miR-148a-3p were quantified in blood plasma and seminal plasma of 65 PCa and 58 BPH patients by digital droplet PCR. The sensitivity and specificity of these microRNAs were determined using ROC curve analysis. The higher expression of miR-182-5p and miR-375-3p in the blood plasma of PCa patients was statistically significant as compared to BPH ($p = 0.0363$ and 0.0226, respectively). Their combination achieved a specificity of 90.2 % for predicting positive or negative biopsy results, while PSA cut-off of 4 µg/L performed with only 1.7 % specificity. In seminal plasma, miR-375-3p, miR-182-5p, and miR-21-5p showed a statistically significantly higher expression in PCa patients with PSA >10 µg/L compared to ones with PSA >10 µg/L. MiR-182-5p and miR-375-3p in blood plasma show higher performance than PSA in differentiating PCa from BPH. Seminal plasma requires further investigation as it represents an obvious source for PCa biomarker identification.The unmet challenge for clinicians in prostate cancer (PCa) management represents its differentiation from benign prostate hyperplasia (BPH) due to the lack of specific diagnostic biomarkers. Contemporary research is directed towards cfDNA from liquid biopsies as potential PCa biomarkers, especially DNA methylation since it plays an important role in prostate cancer development. In the present work, CpG methylation of the LGALS3 gene in cfDNA isolated from blood and seminal plasma of PCa and BPH patients was investigated using pyrosequencing; as well as DNA methylation of LGALS3 in tumor tissue, surrounding non-tumor tissue, and BPH tissue. Liquid biopsy samples were taken from patients with elevated PSA levels before prostate biopsy, who were subsequently divided into two groups (42 with PCa and 55 with BPH) according to the histopathology report. Statistically significant higher cfDNA methylation in seminal plasma of BPH patients was found compared to cancer ones. Still, in .

P-2

Novel biomarker for consensus molecular subtype 2 and stemness in colorectal cancer.

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The consensus molecular subtypes (CMS) classification of colorectal cancer (CRC) represents a robust system for patient stratification based on genomic and transcriptomic profiles that can be potentially applied for the treatment strategy selection. HAKAI is an E3 ubiquitin-ligase (CBLL1) that induces the ubiquitination and subsequent degradation of E-cadherin at cell-cell contacts in a phosphorylation dependent manner, inducing epithelial-to-mesenchymal transition (EMT), tumour progression and carcinoma metastasis. Moreover, HAKAI expression is significantly increased in human colon adenocarcinoma, compared with healthy colon tissues. In our study, we have performed strong genomic assays using big cohorts of colorectal cancer patients to analyse whether HAKAI was associated to any of the CMS. Using robust bioinformatic and machine learning methods, we have analysed HAKAI expression on a large integrated cohort of 1273 primary tumour samples from CRC patients. We have collected genome-wide expression data and clinical survival data for this large CRC cohort to achieve accurate survival analysis and patient risk prediction. Moreover, we used HCT116 colon cancer cells were gown in 2D monolayer and 3D tumoropheres, in an enriched stemness conditions, and viral transduction of shRNA-HAKAI silencing in an inducible system of HT29 were used to analyse expression of stem cancer cells and differentiation RNA and protein genes. We show HAKAI association with CMS2 colorectal cancer together with Myc and Wnt-related genes, helping to elucidate the molecular mechanism by which HAKAI is involved in stemness and its use as potential therapeutic biomarker

P-3

Role of the epitranscriptome and its implication in immune infiltration in cancer.

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Prostate cancer (PCa) is the second most diagnosed cancer in men worldwide and the first in terms of prevalence. Although most patients initially respond to conventional therapies, advanced disease develops resistance to therapy and metastasis. Immune checkpoint blockade (ICB) has emerged in recent years as an effective tumour therapy, but the immunosuppressive tumour microenvironment (TME) of this cancer type makes this therapy ineffective. Epigenetic inhibitors have been shown to be effective in the reprogramming of immune cells in tumoricidal endotype. After analyzing cancer expression databases (TCGA), a significant number of RNA epigenetic regulators (RER) were found to be altered. To explore the interactions between the RERs and the inflammatory status of the tumour in PCa, deconvolution methods were applied, founding a striking inverse correlation between RERs expression and immune cell infiltration with more tumoricidal endotype. These observations lead us to hypothesize that the combined actions of BCI agents together with targeted agents that inhibit aberrant RER may elicit significative immunotherapy responses in cancer.

By using genomic screenings, in silico analysis, CRISPR/Cas9 technology, proteomics, sc-RNA-seq from tumour samples, cell and mouse models and patient samples, we aim to explore the role of RERs in the activation and polarization towards inflammatory or immunosuppressive activity of immune cells associated with cancer, and their influence in the development of PCa.

We have found an inverse correlation between the RNA m7G methyltransferase METTL1 expression and pro-inflammatory macrophage infiltration in human PCa biopsies. We have observed the same results in vivo, in METTL1-deleted tumour mice. In vitro, we observed macrophage repolarization, indicating that METTL1 deletion in the tumour reprograms the tumour microenvironment. Mechanistically, METTL1 tRNA methylation regulates the crosstalk between cells and microenvironment. Furthermore, in our cells we have found that tRNA methylation regulates stress pathways that activates IFNg response.

Our study concludes that METTL1 over-expression in PCa cells induces a pro-regenerative TME, and that its inhibition in PCa may be a promising therapeutic tool to improve immune therapies, as is the case of ICB.

P-4

The Need for Speed – A pan-cancer study on the role of alterations in DNA methylation on proliferation.

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Aberrant DNA methylation plays an important role in gene expression deregulation in most cancers. However, the precise regulatory role of these alterations and their clinical implications are still poorly understood. We perform expression-methylation Quantitative Trait Loci (emQTL) analysis [1] to identify cancer-driving transcriptional networks linked to CpG demethylation in a pan-cancer manner. We performed emQTL by analyzing 33 cancer types from The Cancer Genome Atlas (TCGA). We found cancer-type independent, in cis and trans, the correlations between CpG methylation and gene expression. One of the epigenetically dysregulated transcriptional networks identified was associated with proliferative signaling in cancer and was wired through the binding of transcription factors FOSL1/2 and JUN (forming the AP1 complex) through an intricate connection between enhancer methylation and expression of target genes. In this study, we showed that enhancer demethylation at transcription factor binding regions is linked to transcriptional activation of proliferation-related genes through chromatin looping in various cancer types, thereby suggesting a common architecture of aberrant DNA demethylation revealing the convergence of multiple cancer types.

P-5

The repressor Capicua is a barrier to lung tumor formation driven by KRAS oncogenes.

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CSIC

Mutations in KRAS are well-established as an initiating event in the formation of lung adenocarcinomas. Evidence from genetically-engineered mouse models indicated that signaling via the RAF-MEK-ERK (MAPK) effector pathway is critical for tumor induction. The transcriptional repressor Capicua (CIC) has recently been identified as a critical substrate of ERK kinases in development and disease. In the absence of KRAS/MAPK signaling, CIC binds to specific DNA binding sites and represses transcription of its target genes. In contrast, when the KRAS/MAPK pathway is active, ERK directly phosphorylates CIC and triggers its inactivation leading to de-repression of its target genes. Here, we have explored the role of CIC in KRAS-driven lung cancer formation. CIC inactivation using a conditional loss-of-function mouse model significantly reduced the survival of Kras+/LSLG12V;p53lox/lox (KP) mice infected with Adeno-Cre. These mice, designated as KPCic, developed significantly more tumors, indicating that inactivation of CIC facilitated lung tumor initiation. In addition, we observed that tumors grown in KPCic mice presented reduced levels of allelic imbalance towards to mutant allele. Thus, suggesting that amplification of the mutant Kras allele occurs to effectively inactivate CIC. CIC inactivation facilitated transformation of bronchiolar Club cells to AT2 cell markers, thereby contributing to increased lung tumor initiation and reduced survival. Inactivating mutations in CIC are found in 3% of all human lung adenocarcinomas and rarely co-occur with other known drivers such as KRAS or EGFR. However, CIC mutations coincide with mutations in the tumor suppressor TP53 in 80% of the cases. Indeed, we show that combined inactivation of Cic and Trp53 deletion in mice is sufficient to drive lung adenocarcinoma formation, suggesting that inactivation of CIC could also act as a driving mechanism in human lung cancer.

CIC inactivation also caused resistance to inhibition of the KRAS/MAPK pathway. RNA sequencing revealed de-repression of ETV4 and ETV5 as the main mechanism of tumor growth and resistance upon CIC inactivation and their downregulation resulted in reduced proliferation of lung cancer cell lines. Our data show that loss of CIC function in tumors is equivalent to KRAS activation and creates a selective vulnerability that can be exploited to overcome resistance.

P-6

Shedding light on the methylation of the so-called "dark genome".

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Prostate cancer (PCa) is very heterogenous both at clinical and molecular level, being tailored treatment still highly demanding. As such, better understanding of the mechanisms responsible for PCa onset and progression represents a challenging puzzle.

Non-coding RNAs (ncRNAs) are the most widespread transcripts of mammalian genomes. They are not only post-transcriptional regulators, but are themselves regulated at the RNA level, being chemically modified. Clearer insight of the mechanisms that regulate the function of ncRNAs could be the breakthrough that will implement them as future diagnostic and therapeutic tools.

Hence, our main goal was to tackle whether unveiled RNA methylation profiles may impact and improve PCa clinical decision in a way pioneering and worthy of pursuing. Remarkably, the number of methyl marks found on RNA is about seven times greater than those at DNA level. Nevertheless, our ability to decipher these altered nucleobases was significantly delayed until recent years when the explosion in

high-throughput methods and further mechanistic studies revealed the roles and regulation of RNA methylation in many fundamental and disease-associated biological processes.

Here, we portrait the different methyl marks in ncRNAs as relevant molecular fingerprints of PCa. RNA methylation at position N6 in adenosine (m6A) is one of the most versatile chemical alterations found in the prostate epitranscriptome. Specifically, we found that VIRMA, a protein of the multi-component writer complex, is essential for the maintenance of m6A in PCa and its depletion reduced global m6A levels causing malignancy arrest. The removal of specific m6A marks from investigated long ncRNAs reduced transcript stability and abundance.

Additionally, ribose methylation at position 2'-O (2-O-methylation) is the most common modification in abundant non-coding RNAs, such as ribosomal RNA (rRNAs) and methylated ribosomal profiles are different among malignant and non-malignant prostate tissue. Indeed, methylated rRNA signature was an additional layer of molecular characterization of PCa heterogeneity, useful to improve PCa patient stratification and clinical management.

In sum, we showed that aberrant RNA methylation can modulate gene expression, leading to translational reprograming, cancer cell survival and proliferation. In the future, we should consider this as important factors to pave the way of precision medicine.

P-7

Mapping RNA modifications in long-lived and cancer-resistant naked mole-rat.

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Naked mole-rat cells produce fewer aberrant proteins compared to mice [1, 2]. This is due to higher protein synthesis fidelity, which has been shown to contribute to increased lifespan. These animals live 10 times longer than other rodents of a similar size, without developing age-related diseases, such as cancer. All RNA components of the protein synthesis machinery (mRNA, rRNA and tRNA) are decorated with chemical modifications. These modifications are necessary for biogenesis, stability and function of each RNA. We wanted to know how naked mole-rats sustain an elevated translational fidelity by mapping RNA modifications in this remarkable rodent for the first time. To map all RNA modifications present in rRNA we directly sequenced large RNA from mouse and naked mole-rat livers using Nanopore-sequencing, side-stepping the standard cDNA conversion step, which is inadequate for detecting most modifications. Here we show that the naked mole-rat's small ribosomal subunit (Rn18S) carries a unique array of RNA modifications not present in mice. By comparing the mouse modified Rn18S with an unmodified control we could attribute over 90% of Nanopore current deviations to known RNA modifications using Yanocomp [3]. Positions of ribosomal RNA modifications are well-conserved between species and few exceptions are known. Unexpectedly, we found five new putative RNA-modification regions in the naked mole-rat Rn18S. Base-mapping errors in the underlying sequences suggest that the modifications could be pseudouridines. Our results demonstrate that the naked mole-rat's ribosome has a unique composition of RNA-modifications that could contribute to elevated translational fidelity. Learning how the stress-tolerant naked mole-rat produce error-free proteins can be used to reprogram human cells to age healthier.

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P-8

Epigenetic regulation of EMT in Breast Cancer.

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One of the hallmarks driving carcinogenesis are genetic and epigenetic alterations, where aberrant DNA methylation plays a big role and contributes to driving carcinogenesis in several cancer types, including breast cancer as one of the most common in women. One bioinformatic approach to identify differentially active pathways in cancer potentially under epigenetic control is by using expression-methylation Quantative Trait Loci (emQTL), which performs an integration of genome wide CpG-methylation and gene expression. This has previously revealed a bicluster highlighting both changes in epithelial-to-mesenchymal transition (EMT) and variations in fibroblast infiltration in breast cancer. Induced EMT in the breast cancer cells make them detach from neighboring cells and acquire migration features, initiating invasiveness and metastasis. As a big component within the tumor microenvironment can Cancer-Associated Fibroblasts (CAFs) induce EMT in the cancer cells through paracrine signaling.

In this project, we aim to induce EMT in vitro with the two ER+PR+ breast cancer cell lines MCF7 and T47D by incubating them with a conditioned media collected from the CAFs, and assess molecular alterations using RNA-seq, ATAC-seq and Illumina EPIC to investigate gene expression, open chromatin state and DNA methylation, respectively.

P-9

Integration of DNA Methylation and Gene Expression in Lung Adenocarcinoma: Epigenetic Regulation, Patient Classification, and Prognosis.

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Aberrant DNA methylation accompanied by aberrant gene expression is a hallmark feature of many cancer types, including lung cancer. To investigate how these events are associated and what disease-associated pathways are under epigenetic regulation, we perform genome-wide expression-methylation Quantitative Trait Loci (emQTL) analysis by integrating DNA methylation and gene expression data obtained from lung adenocarcinoma (LUAD) patients. Grouping emQTLs through biclustering led to the discovery of biological pathways associated with immune cell infiltration, proliferation, and hormone signaling. Of particular interest, we found the hormone-related CpGs to be enriched in enhancer regions and the binding sites for transcription factors linked to hormone-related signaling such as FOXA1 /2, FOSL1/2, SMAD3, and JUN. The identified hormone-related genes and CpGs were connected through chromatin interaction loops, implying the implication of DNA methylation in epigenetic regulation through enhancer-promoter interactions. Moreover, by performing unsupervised hierarchical clustering of the emQTL-genes and CpGs, we identify and validate subclasses of patients with different prognoses based on the expression pattern of hormone-related genes. The identified molecular subclasses partially overlapped with known molecular subtypes in LUAD, providing additional information in tumor classification not captured by previous studies using transcriptomic data independently from DNA methylome.

P-10

BET bromodomain activity is required for basal stem cells differentiation of human airway epithelium.

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Airway epithelium is at the frontline between the environment and our inner body. To understand this epithelial function is fundamental to prevent and combat pathological states, including infections, allergic reactions or cancer development. In a pharmacological screening looking for stem cell factors that could help developing experimental models to study the airway epithelium, we found that 4 signaling pathways affected airway epithelial morphology in a new human airway epithelium model system. Furthermore, in a secondary screening focusing on epigenetic factors, we found and confirmed that only bromodomain and extra-terminal domain (BET) protein activity from the bromodomains family blocks airway basal stem cells (BSCs) pseudostratification. Then, to analyze if this early epithelial phenotype could affect epithelial regeneration, we tested those inhibitors during BSCs differentiation. In our experimental conditions, we found that basal stem cells differentiation towards multiciliated or secretory cells is completely blocked in the absence of BET proteins activity. Given the importance of BET proteins reading the histone acetylation status, we anticipate that specific epigenetic signals are crucial for airway epithelial regeneration.

P-11

The Role of N6-Methyladenosine in Mediating Drug Resistance of Human Hepatocellular Carcinoma.

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N6-methyladenosine (m6A) methylation is a dynamic epitranscriptional modification controlled by the combined action of specific writers, erasers and readers (effectors) markedly impacting RNA metabolism. m6A has been reported to participate in the development of hepatocellular carcinoma (HCC). However, its role in HCC drug resistance remains unclear.

Reanalyzing data of a pharmacogenomic study in HCC we identified a number of m6A effectors whose expression was significantly associated to drugs resistance or sensitivity. We hypothesized that we may revert cell resistance to the drugs by altering the fate of modified RNAs through the perturbation of specific m6A effectors. Selecting three different HCC cell lines (HepG2, Hep3B, Snu475), we shortlisted 3 different combinations of m6A effectors and drugs. We demonstrated that the knockdown (through RNAi and CRISPR-I) of specific effectors (ALKBH5, VIRMA, YTHDF2) synergizes with various chemotherapy drugs, sensitizing in vitro models of HCC against these drugs and reducing their viability. Furthermore, ALKBH5 is markedly and consistently upregulated following the drug treatment in all the tested cell lines, suggesting a direct involvement of the effector in the chemotherapy drug response pathway. Finally, the knockdown of ALKBH5 leads to changes in m6A bulk levels in the different cell lines, suggesting that its role is related to its catalytic function in the m6A machinery.

We are currently characterizing through Nanopore direct sequencing of native RNA whether m6A-dependent alterations of the RNA metabolism are central in the drug resistance. Ultimately, these data might disclose novel molecular mechanisms through which epitranscriptional alterations impact on drug resistance and shape HCC aberrant transcriptional programs.

P-12

Defining stages in cardiomyocyte differentiation through AI classification.

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Cell differentiation is a key step in multiple patho-physiological processes like development or cancer. This is often considered a multi-stage event, and in some cases these sub-steps are not fully clear. In other cases, a set of stages has been defined but the identification relies either in techniques like scRNA-seq or in a priori known markers of the stages. Therefore, novel tools to detect and understand the cell differentiation processes are needed. Here we show how an AI-based method that uses light microscopy (marker-free) images can be utilized to describe multiple stages in a known cell differentiation. Specifically, we used hiPS to cardiomyocyte differentiation in 2D to characterize the different events that happen through this cell process. We developed an AI (deep learning) method that quantify the differences between a set of cellular images and a known library of hIPS, and then a statistical framework to obtain the score threshold between different stages. In summary, we present a new method that is able to characterize the stages during a complex cellular process, and this might be applied to other relevant events like tumorigenesis.

P-13

Changes at epigenomic level influence fibroblast migration and proliferation in mucopolysaccharidosis patients.

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Mucopolysaccharidoses are rare diseases in which there is an accumulation of glycosaminoglycans (GAGs), that are a class of polysaccharides present in all mammalian cells. These molecules are present not only on the cellular membrane, but also in the intracellular milieu and extracellular matrix. In the last few years, it has been shown how GAGs have a profound role in multiple physiological and pathological processes.

The objective of this study was to test if there were differences between the dermal fibroblasts from healthy people (control) and from patients suffering distinct types of mucopolysaccharidosis. Analysing the results, we showed changes caused by the accumulation of different GAGs in different genes involved in some processes such as proliferation or migration that are concomitant to molecular alterations in the transcriptomic profiles of the cells derived from patients. Furthermore, the RNA levels were modified after different epigenetic treatments. Even though further studies are needed in which we will explore the molecular mechanisms underlying these changes, these results suggest that human dermal fibroblasts from patients that are suffering different mucopolysaccharidoses have altered properties regarding to important physiological processes for the cell biology and cancer.

P-14

Triple-negative breast cancer tumors from young Black women show a distinct DNA methylation landscape.

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Introduction: Triple-negative breast cancer (TNBC) is characterized by the lack of estrogen and progesterone receptors and the absence of HER2 overexpression. This subtype shows a reduced disease-free survival compared to the other subtypes across all the ethnic groups. However, clinical data indicates that Black women have a higher prevalence of TNBC than White women, especially at young ages. Furthermore, Black patients have a worse response to neoadjuvant chemotherapy treatment, suggesting a more aggressive disease affecting this group of patients.

Methods: We integrated data from two studies (GSE39004 and TCGA; n=167 TNBC patients) to compare clinical factors and DNA methylation (DNAm) between Black and White patients. We identified genomic regions with the most variable DNAm levels across the groups and computed the Spearman's rho distance between young (< 50 years old) and older Black and White TNBC patients. Furthermore, we employed the Wilcoxon test to identify genomic regions specifically differentially methylated in young Black patients.

Results: We found DNAm differences between the four groups. Interestingly, older Black patients have a DNAm landscape that is more similar to White patients of any age than young Black patients, suggesting a unique epigenetic program affecting pre-menopausal Black patients. Furthermore, 1,035 CpG sites were specifically differentially methylated in TNBC from young Black patients compared to the other groups. Interestingly, when using the 500 most variable genomic regions, TNBC tumors from young and older Black patients clustered together, suggesting the presence of a core set of genomic regions whose DNAm level is associated with ethnicity.

Conclusions: We found that young Black TNBC patients have a distinct DNAm landscape, which might account for the higher incidence and worsened outcome noted in this specific patient cohort. These results highlight the need to explore ethnicity- and age-related epigenetic variations in TNBC patients to further define molecular determinants of higher prevalence and response to treatments.

P-15

Chromatin structure imparted by histone H1 facilitates m6A deposition on nascent RNAs that need to be restricted in pluripotent cells.

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N6-methyladenosine (m6A) is one of the most prevalent internal modifications on mammalian RNA molecules, regulating multiple aspects of its fate such as translation, splicing, or decay, and is thus involved in crucial biological processes in physiology and pathology. m6A is installed co-transcriptionally by different factors of the writer complex that bind to chromatin and interact with RNA polymerase II (RNAPII) during transcription elongation. To evaluate how transcription dynamics impacts on m6A deposition we have addressed RNAPII elongation rates and nascent RNA abundances in mouse embryonic stem cells with altered chromatin configurations due to reductions in linker histone H1 content. We found that coding genes transcribed at slow rates are preferentially m6A methylated and display unique signatures at their promoter regions, namely high levels of histone H1, marks of bivalent chromatin, and low RNAPII pausing. These genes are also highly susceptible to m6A loss upon histone H1 reduction compared to those transcribed at higher velocities. In addition, in the absence of histone H1, there is an increase in the transcription of non-coding RNAs, together with reduced levels of m6A modification leading to their accumulation on chromatin and causing replicative stress due to replication-transcription conflicts. Altogether, our findings unveil an unexpected role of histone H1 in the m6A regulatory axis and highlight the crosstalk between chromatin structure and the deposition of this mark to regulate RNA metabolism at specific transcript classes and ensure genome performance.

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P-16

Proteomic-wide association studies to decipher Colorectal Cancer predisposition.

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Colorectal cancer (CRC) is one of the most prevalent tumors and an important health burden. Although it presents a high heritability, current genomic strategies (GWAS) have only been able to identify a small fraction of the genomic loci involved in CRC susceptibility. Nevertheless, calculations estimate we would need GWAS with sample sizes in excess of 1M to uncover 80% of the heritability. Therefore, other approaches are needed to overcome this limitation. In this regard, association studies of different molecular phenotypes have emerged as a powerful tool to decipher the heritability of CRC. Phenotypes such as gene expression, methylation or protein levels can be tested for association.

In this work, we have gathered the publicly available data on protein QTLs (pQTLs) to perform proteomic-wide association studies of CRC. For this, we have converted the pQTLs data to protein level models that can be used in the PredictDB pipeline to assess association with CRC susceptibility. We have used 13 datasets amounting 29196 samples from blood, plasma and serum-measured proteins, as is the only tissue of interest with available data and discovered that the genetically-predicted levels of 26 proteins are related to CRC risk. This will provide us not only with important insight into how CRC risk is mediated, but also the cellular pathways implicated, which could yield relevant information to launch chemopreventive strategies.

P-17

Analyzing the role of Non-SMC Condensin I Complex Subunit H in breast cancer development and evolution.

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Background: Non-SMC Condensin I Complex Subunit H (NCAPH) is one of the three non-SMC that participate in mitotic chromosome architecture and segregation. Previous studies have reported that abnormal expression of NCAPH is involved in the progression of several types of human cancer, including breast cancer. In this study, we delve into the role of NCAPH as a marker of breast cancer evolution and its role in breast cancer pathogenesis.

Methods: We analyzed the differential gene expression of NCAPH in tumor and normal tissue with the TNM plot and the prognosis with the Kaplan-Meier plotter. We evaluated the expression of NCAPH by immunohistochemistry in a cohort of breast cancer patients and their association with the disease's evolution. We generated transgenic mice that overexpressed NCAPH under the MMTV promoter, and we crossed them with MMTV-Neu mice to generate double-transgenic mice. Also, the effect of NCAPH overexpression was evaluated in different breast cancer cell lines.

Results: NCAPH is overexpressed in tumors versus normal mammary tissue. Moreover, NCAPH overexpression is correlated with poor prognosis in humans' luminal A and luminal B breast cancer. Immunohistochemistry studies confirmed the poor evolution in luminal A tumors with high levels of NCAPH expression in a new cohort of patients. In vitro, high levels of NCAPH expression were associated with high viability, high proliferation, less apoptosis, high genomic instability, more migration, and stronger pAKT expression. In vivo, MMTV-Neu/NCAPH double-transgenic mice developed more tumors with more aggressiveness.

Conclusions: NCAPH is a candidate gene overexpressed in invasive ductal carcinoma, and NCAPH expression correlates with poor prognosis in different types of breast cancer.

P-18

7-Guanosine tRNA methylation regulates Prostate Cancer progression through protein translation reprogramming via tRNA-derived fragment biogenesis.

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Prostate Cancer (PCa) is the most common cancer and the third cause of death by cancer in European men. Although most of the patients respond to hormone deprivation therapy, many patients develop Castration Resistant Prostate Cancer (CRPC) with limited treatment options. This progression is due to the existence of pre-existing cancer cell sub-populations resistant to conventional treatments and with high self-renewal capacity, leading to tumour regeneration and therapy resistance. Recent data has shown that self-renewal and stress resistance are controlled by RNA modifications, and manipulation of the epitranscriptome may be a potential therapeutic target to specifically eliminate those cancer cells resistant to conventional treatments. Thus, we aim to decipher the epitranscriptome in PCa in order to implement novel therapeutic strategies to eliminate cancer cells with high self-renewal capacity.

In silico analysis of sequencing data from different databases demonstrated that the tRNA methyltransferase METTL1 was overexpressed in primary and advanced tumours, being increased expression correlated with poor prognosis. Altered expression of the methyltransferase was confirmed by qPCR and WB of primary tumours patient samples from Basurto Hospital and samples from a PCa mouse model. For functional characterization of METTL1 role, cells over-expressing, silenced and knocked out for METTL1 using CRISPR/Cas9 were generated in PCa cell lines. Lack of METTL1 resulted in increased tRNA fragmentation which lead to a dysregulation of the normal translatome and global translation inhibition. As a result, METTL1 deletion resulted in impaired cell proliferation and self-renewal capacity in cell cultures and reduced tumour formation capacity in xenografted model and in PCa murine model. In addition, cells lacking METTL1 exhibited dysregulated redox homeostasis and increased DNA damage, resulting in increased stress sensitivity.

Our study concludes that METTL1 is overexpressed in PCa and higher expression correlates with poor prognosis. METTL1 is essential for m7G deposition in tRNAs and its deletion leads to protein synthesis alterations, which results in a deregulation of essential cellular processes as proliferation and self-renewal. Whether METTL1 can be used as a therapeutical target needs further validation.

P-19

Functional characterization of a m1A methyltransferase that modifies the 28S rRNA.

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Ribosome synthesis is a multi-step process during which the transcription, modification, folding and processing of the rRNA precursor are coordinated with the incorporation of ribosomal proteins to produce the two ribosomal subunits. This process is driven by more than 200 ribosome biogenesis factors (RBFs) which, along with the pre-rRNA and ribosomal proteins, form different preribosomal complexes. The most abundant and stable preribosomal complexes are known in great detail, both at the compositional and structural level, thanks to the revelation of their 3D structures by cryo-EM. However, there are preribosome intermediates that still remain obscure. Indeed, many RBFs have not been found associated to any of the

known preribosomes and, therefore, it is uncertain when and how they exert their functions. For many of those RBFs, their functional relevance for ribosome maturation is not well understood. One example is RRP8, a methyltransferase that catalyzes the m1A1322 of the 28S rRNA. We have generated a set of HeLa-derived cell lines that endogenously express GFP-fused versions of RRP8 and other RBFs, and carried out GFP-Trap pull-down experiments under conditions that preserve nucleolar preribosomes, coupled to mass spectrometry analysis, in order to identify the precursors that contain RRP8. We have also ascertained the impact of the knockout and knockdown of RRP8, and the importance of its methyltransferase activity, on the production of ribosomes. The obtained results reveal that RRP8 is an integral component of an as yet uncharacterized preribosomal intermediate and that, although this protein only modifies the rRNA of the large subunit, it plays an important role in the assembly of both ribosomal subunits.

P-20

N6-Methyladenosine RNA Modification and Its Regulatory Proteins in Renal Cell Carcinoma.

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Recently, it has been found that RNA methylation can promote the progression of renal cell carcinoma (RCC). The active regulation of methylation of N6-adenosine (m6A) and the dynamic effects of m6A regulators determine the overall impact of m6A on cells and tissues of renal cell carcinoma (RCC). Nonetheless, most of the studies focused on components of the methyltransferase complex, while the role of erasers has been seldom explored in RCC. Furthermore, the few works available on m6A are "ccRCC-centric", the most common RCC subtype. Herein, we assessed the expression profiles of m6A regulatory proteins in RCC and their clinical relevance as potential biomarkers.

In silico analysis of The Cancer Genome Atlas (TCGA) dataset was used for evaluating the expression of the m6A regulatory proteins among RCC subtypes. Expression levels of m6A players were also assessed in a wide panel of RCC cell lines. ALKBH5 and FTO, two m6A eraser proteins, were evaluated in a series of primary RCC (n= 120) and 40 oncocytomas by quantitative PCR and immunohistochemistry.

Detailed evaluation of the global expression of all regulators demonstrated that erasers (FTO and ALKBH5) are highly expressed in RCC compared to writers (METTL3/14, WTAP and VIRMA), specifically in clear cell RCC (ccRCC). Additionally, high FTO transcript levels revealed a protective effect for overall survival in ccRCC and papillary RCC patients, independently of other relevant clinical and pathological variables. Further validation of the clinical relevance of m6A eraser proteins in IPO-Porto's patient cohort depicted higher immunoexpression of FTO and ALKBH5 in ccRCC comparatively to the other RCC subtypes and oncocytomas.

Interestingly, similar m6A levels were found among the different RCC subtypes and oncocytomas. These observations may be explained by the fact that immunohistochemistry only allows for semi-quantitative assessment of m6A at the global level, whereas m6A modification may affect different transcripts (with distinct implications) among the various renal cell tumor subtypes.

We conclude that altered expression of m6A RNA demethylases is common in RCC and seems to be subtype specific. Specifically, FTO and ALKBH5 might constitute new candidate biomarkers for RCC patient management, aiding in differential diagnosis of renal masses and prognostication.

P-21

Identifying regulatory mechanisms of the histonemethylase SMYD3 in breast cancer.

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Aim; In breast cancer, metastasis occurs in almost 30% of patients and fatality is often ascribed to metastatic events. Cells able to leave the primary tumor undergo epithelial-mesenchymal-transition (EMT), exhibit mesenchymal features, and disseminate metastasis throughout the body. EMT has been associated with cancer stem-like properties, including tumor- initiating capacity to maintain primary tumor growth in breast cancer. The most accepted model to explain cancer metastasis suggests that a small subpopulation of cancer cells acquires cancer stem-like cell (CSCs) traits, exhibits mesenchymal characteristics, and migrates away from the primary tumor site and progresses to distant metastatic sites. Many histone methylases and demethylases are involved in regulation of CSC. The methyltransferase SMYD3 plays a key role in the regulation of EMT. SMYD3 can methylate also non-histone proteins, which are involved in cancer cell proliferation and to modulate transcriptional response to promote transcriptional reprogramming, tumor transformation and progression. In this study we investigated the role of SMYD3 in maintenance of CSC traits and whether SMYD3 depletion or pharmacological blockade may restrict CSC-like properties in breast cancer cells.

Meterial and methods; We isolated ALDH1+/CD44+/CD24- CSCs and ALDH1-/CD44-/CD24+ non-CSCs from MDA-MB-231 and other breast cancer cell lines by using Fluorescence Activation Cell Sorting (FACS) and employed Western blot, RT-PCR and ChIP analysis to characterize SMYD3 role in CSC, using CRISPR/Cas9 SMYD3 KO cells. In vitro functional assays such as spheroid formation efficiency, wound healing assay, pharmacological blockade of SMYD3 were also performed.

We found that SMYD3 is upregulated in breast CSCs compared to non-CSCs. Also, silencing of SMYD3 suppresses CSCs percentage and leads to inhibition of breast cancer cell spheroid formation and motility. Remarkably, pharmacological blockade of SMYD3 partially reduce mesenchymal and stemness of MDA-MB-231 cells.

Results; Our findings suggest hat SMYD3 correlates with regulation of the EMT process in breast cancer and plays a role in metastasis formation. Overall, our findings provide new insights into the regulatory role of SMYD3, by targeting genes relevant for cancer stemness maintenance and SMYD3 inhibitors may become a potential therapeutic strategy to target breast cancer stem cells.

P-22

Metascreen / A modular tool for building pipelines to design and analyze drug combination screens.

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The development of targeted therapy has revolutionized the treatment of cancer. However, with a few notable exceptions, such as treatment of CML with Imatinib, the vast majority of single-drug targeted therapies ultimately fail, often due to acquired resistance. One strategy to delay or prevent drug resistance is to use drug combinations. The potency of drug combinations is highlighted by the classic example of combination therapy for HIV patients, which turned a deadly disease into a chronic condition for the vast majority of patients. Systematic identification of synergizing drug combinations poses unique bioinformatics challenges, such as the need for pipelines that can be used to design such screens, perform quality control on the data, and generate data files that can be analyzed by synergy-finding bioinformatics applications.

Here, we present metascreen, which is an open source, end-to-end modular tool available as an R-package for the design and analysis of drug combination screens. The tool allows for a customized build of pipelines through its modularity and provides a flexible approach to quality control and data analysis. metascreen is adaptable to various experimental requirements with an emphasis on precision medicine. It can be coupled to other R packages, such as bayesynergy, to identify synergistic and antagonistic drug interactions in cell lines or patient samples. metascreen is scalable and provides a complete solution for setting up drug sensitivity screens, read raw measurements and consolidate different datasets, perform various types of quality control, and analyze, report and visualize the results of drug sensitivity screens.

P-23

Understanding splicing vulnerabilities of MYC activation in cancer cells.

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Upregulation of the transcription factor Myc in a large number of human cancers is associated with poor prognosis (Chen et al., 2018). Interestingly, Myc-overexpressing cancer cells display increased sensitivity to splicing inhibitors targeting the core splicing factor SF3B1 (Hsu et al., 2015). It has been proposed that Myc overexpression increases transcription of a large number of targets and this induces stress on the splicing machinery such that these cancer cells display a synthetic lethal (SL) phenotype when Myc upregulation is combined with SF3B1-targeting drugs (Koh et a., 2015). This project aims to characterize the molecular events and mechanisms that make Myc-overexpressing cancer cells particularly vulnerable to splicing inhibitors.

We are studying this phenomenon using an immortalized epithelial cell line (h-TERT RPE MYC-ER p53KO) which allows for long-term, inducible MYC activation, and the SF3B1-targeting drug Pladienolide B (PLAD-B). Using this model, viability and clonogenic assays reproduced the SL phenotype upon MYC activation and splicing inhibition using PLAD-B. We have isolated RNA from cells treated with PLAD-B (or not) for different times, in the presence or absence of induced Myc. Our aim is to correlate changes in alternative splicing induced at different time points, determined by RNA-seq, with the SL phenotype of the cells, determined using viability assays. So far we have obtained a set of alternative splicing changes that behave differently under SL conditions compared to Myc activation or splicing inhibition alone and that affect exons known to be essential for cancer cells. Contrary to expectation, promotion of the pro-apoptotic isoform of MCL1 (MCL-1S), which has been proposed to be a major mediator of apoptotic effects induced by PLAD-B treatment (Kashyap et al., 2015) cannot explain the SL phenotype. We are currently validating the relevance of additional splicing changes and we will evaluate their biological and pathological roles and their potential therapeutic value against Myc-overexpressing cancer cells.

P-24

Glioblastoma-specific enhancer elements affected by non-coding single nucleotide variations.

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Gliomas are primary brain tumors subdivided into low-grade gliomas (LGG) and high-grade gliomas or glioblastomas (GBM). GBM has a higher recurrence rate and a worse prognosis than LGG. Besides distinct clinical behavior, LGG and GBM exhibit significant molecular differences. Epigenetics mechanisms involving DNA methylation and histone modifications play a significant role in cancer progression and resistance traits. These changes are due, in part, to changes in cis-acting gene regulatory elements (GREs), such as enhancer elements (EEs), which can modify the expression pattern of proximal or distal gene networks. Non-coding mutations affecting EEs have shown a significant impact on cancer initiation and progression. Here, we proposed to identify GBM-specific EEs with non-coding mutations and characterize the affected transcription factors binding sites.

We integrated ATAC-seq data from GBM (n=9) and LGG (n=11) patients to explore differential chromatin accessibility and functional epigenetic maps generated by the FANTOM5 project to identify GBM-specific dynamic EEs. The binding of multiple transcription factors (TF) to these GREs was identified using the ENCODE ChIP-seq data. Then, we overlapped whole-genome sequencing data from GBM patients (SweGBM-1 cohort, n=38) to identify non-coding mutations affecting GBM-specific EEs. Finally, the Factorbook server was employed to estimate differential TF binding affinity between wild-type and mutant GBM-specific EEs.

We identified 30 GBM-specific EEs which bind multiple TFs and considered seven EE candidates for downstream analyses. The candidate EEs have GBM-related genes in a window of 2Mb. The candidate EE_3, located in chromosome 12, has a recurrent mutation in three GBM patients and it is near PRMT8, FOXM1, and TEAD4, three genes associated with GBM resistance and aggressiveness. Moreover, the candidates EE_1 and EE_2 showed non-coding mutations in two patients each. EE_1 is near three GBM-associated genes (VEGFA, RUNX2 and CDC5L) and EE_2 has seven neighboring genes (PUS7, SRPK2, PIK3CG, NAMPT, RINT1, HBP1, PRKAR2B). Currently, mutations affecting each EE are being tested in an independent cohort of GBM patients (n=48). Completion of this study will provide important clues about the mechanisms underlining pathological non-coding genetic alterations in patients with GBM.

P-25

Integration of ChIP-Seq and RNA-Seq data to elucidate epigenetic responses in breast cancer patients treated with aromatase inhibitors (the NEOLETEXE trial).

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Breast cancer (BC) is the most common invasive cancer in women accounting for about 25% of all cancers. The majority of tumors (70%) are estrogen receptor positive and suitable for anti-estrogen therapy which includes medication with aromatase inhibitors (AIs). Two of such inhibitors; letrozole (LET) and exemestane (EXE) show at least temporary 'lack of cross-resistance' and can be used sequentially. We have previously performed and integrated whole genome DNA sequencing with whole transcriptome RNA sequencing data from the same tumors, to identify the cellular characteristics of the subclones, identifying changes in signatures such as estrogen signaling, epithelial-mesenchymal transition and interferon signaling. In the present follow-up analysis we attempt to elucidate (epi)genetic responses caused by these drugs and discover factors contributing to the clinical outcome in the two arms of the project (EXE-LET and LET-EXE). We used ChIP-Seq (H3K4me2 histone) and bulk RNA-Seq data from up to three time points from tumors in 12 patients. Analysis was done using Gencode v41 annotation and hg38.13 genome. ChIP-Seq peaks were called with sicer2 and inputs/controls specific for the patients giving us 1-30k peaks per sample and 360k unique peaks in total. Peak qualities were assessed using deeptools. Mapping of RNA-Seq was done with STAR. About 15k protein coding genes are expressed. Among the top 100 highly expressed genes we found genes typical for the connective and immune tissues. Combining gene expression and ChIP-Seq permitted selection of high quality peaks (160k overlapping, 10k within 5kb upstream located closely to the genes expressed above the threshold. Through these analyses, we attempt to identify the intra-tumor mechanisms of adaptation to Letrozole and Exemestane and possibly identify biomarkers that can allow us to detect responders to non-responders, and to dissect the heterogeneity and individual response to treatment with different AIs.

P-26

Decoding chromatin accessibility signatures through targeting DOC1 and NuRD in oral squamous cell carcinomas.

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Head and Neck Squamous Cell Carcinoma (HNSCC) represents the sixth most commonly diagnosed cancer worldwide where five-year survival rates remain one of the lowest. Currently there are limited options for biomarker-guided, molecular-targeted therapies in SCC. Thus, we need to advance our understanding of oral cancer aetiology and development before further improvement can occur. We discovered that the loss of a chromatin-associated factor Deleted in Oral Cancer 1 (DOC1) in oral SCC of the tongue contributes to the development of this cancer.

We hypothesise that the altered function of chromatin remodelling complexes in oral SCCs can be exploited with novel treatment strategies. We examined the function of DOC1 and other chromatin components in OSCC cell lines as well as determine whether disruption of chromatin-associated DOC1 in patients can be targeted with epigenetic modifying agents (EMAs). In order to understand the molecular mechanism underpinning DOC1-mediated chromatin modulation and cancer progression, we used relevant experimental approaches and utilised emerging single-cell (sc)ATAC-seq technologies to map, predict as well as validate the function of chromatin-associated factors and accessibility to the epigenome.

(In)accessible chromatin organization across the (epi)genome echoes a crosstalk of interactions where enhancers, promoters and chromatin-binding factors cooperatively regulate gene expression. Yet little is known about the molecular basis and regulatory mechanisms underlying alterations in the activities of nucleosome remodelers and their roles in cancers. We investigated the role of DOC1-NuRD in initiating (in)accessible remodelling and address the elusive link between chromatin remodelers and cancer, thus potentially discovering novel therapeutic approaches and druggable targets. We will illuminate how chromatin (in)accessibility delineates an inventory of regulatory regions within the (epi)genome and how these epigenetic features are dynamically regulated to control gene expression. Our single cell chromatin accessibility analyses reveal targetable pathways in OSCCs and establish the role of DOC1-NuRD in global nucleosome organization.

P-27

Understanding hormonal treatment resistance in ER+ breast cancer using single-cell chromatin accessibility assay.

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The estrogen receptor positive (ER+) subtype is the dominant contributor to global deaths from breast cancer, with subsequent hormonal treatment resistance acting as the major contributor to patient relapse and eventual death. ER+ breast tumours undergo genome-wide epigenetic alterations, including enhancer methylation that results in oncogenic estrogen signalling (Fleischer et al., 2017), that are crucial in the development of resistance to hormone therapy. Understanding the molecular mechanisms that drive hormone treatment resistance is of great importance for the survival of this group of breast cancer patients.

Single-cell ATAC-seq (scATAC-seq) allows for the systematic exploration of the evolving epigenetic landscapes of both tumour and non-tumour cell populations of a biopsy, offering novel insight into cell biology, disease etiology and treatment response. We aim to characterise the intrinsic and acquired epigenetic properties of responding and non-responding tumours from breast cancer patients undergoing hormonal treatment.

Here, we will present ongoing optimization and test runs of nuclei isolation from frozen breast tumour tissue, scATAC-seq sample library preparation for the 10X Genomics system and preliminary scATAC-seq data.

P-28

Role of cytosine-5 methylation of ribosomal RNA in cell cycle control.

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5-methylcytosine (m5C) is one of the most well-known post-transcriptional modification in RNA. This mark can be found mainly in transfer RNA (tRNA) and ribosomal RNA (rRNA) and is mediated by DNMT2 and NSUN family members. Recently it has been shown that m5C on tRNAs regulates stem cell functions and stress responses, and its inhibition specifically eliminates cancer initiating cells, suggesting that RNA methylation may regulate essential cellular and physiological processes and its dysregulation may lead to critical pathological consequences such as cancer.

In contrast to tRNAs, the functional role of m5C in other RNAs in which this mark is prevalent like rRNA has not been deeply studied in mammals yet. NSUN5 is a m5C methyltransferase that methylates position C3872 of 28S rRNA, located at the interphase between small and large ribosome subunits and its depletion alters global protein synthesis and translation fidelity. Here we show that NSUN5 loss-of-function in mammalian cells leads to lower proliferation rates and altered cell cycle progression throughout G2/M phase. Mechanistically, reduced m5C deposition at C3782 induces a translational rewiring that favors the synthesis of mitotic regulators. In addition, we have found that NSUN5 is post-translationally modified during G2/M, which leads to an alteration of its stability. Our data suggest that NSUN5 expression needs to be modulated along cell cycle to allow proper translation and, eventually, correct progression through this important cellular process.

Cell cycle dysregulation is a key event that governs aberrant proliferation of cancer cells. Given this, cell cycle has been a target for drug development for many years, with promising preclinical and clinical data. NSUN5 is overexpressed in several cancers such as advanced metastatic PCa, glioma, diffuse large B-cell lymphoma, and liver hepatocellular carcinoma, among others. Thus, uncovering the molecular mechanisms behind NSUN5 control over cell cycle could provide new therapeutic targets for cancer treatment.

P-29

Epigenome-wide association study for radiation late severe toxicities.

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Objective and purpose

To identify DNA methylation patterns associated with the presence of severe toxicities after radiotherapy treatment in prostate cancer patients.

Methodology

Sixty-four prostate cancer patients -32 cases and their matched controls- treated with three-dimensional conformational radiotherapy (3D-CRT) at the Clinical University Hospital of Santiago de Compostela (Spain) between 2014 and 2018, were selected. Written informed consent was obtained for each patient. Cases developed some late toxicity CTCAE grade >= 2 after twelve months of starting radiotherapy. Methylation study was performed over 865,918 probes with the chip MethylationEPIC Bead Chip. Patients with a CpG island detection underneath 96% were removed. Low-quality probes (< 3 beads in > 5% of samples) or with detection P-values > 0.01 were filtered out. Non-CpG, non-autosomal, multi-hitting or containing polymorphisms probes were also eliminated. Type I and type II probe distributions were normalized with a Beta-mixture quantile normalization (BMIQ) method. Batch effect was identified with singular value decomposition (SVD) and corrected through a ComBat approach. EpiDISH tool combined with the Robust Partial Correlation (RPC) algorithm was implemented to estimate Cell type proportions (CTp). Differentially methylated positions (DMPs) were identified by implementing limma package that allowed to detect differentially methylated regions (DMR) with DMRcate package, in both cases with age and CTp as covariates. Unadjusted p-values were corrected using the Fisher approach. Methylation data were corrected by the technical variables: sample freezing before RNA extraction and batch effect.

Results

303 DMPs were differentially methylated in one of the groups. We detected 28 DMRs of which 10 were significantly associated with a F < 0.05. Of these, 6 DMRs were located within the upstream or genetic region of 6 genes (HKR1, ABCA13, STK32C, ZAP70, SOSTDC1 and SHANK2).

Conclusions

We have identified six genes with DMR associated with the presence of side effects in patients with prostate cancer after undergoing radiotherapy treatment. The differential expression of two of them -ABCA13 and ZAP70- has been related to the efficacy of radiotherapy in other types of cancer. Our results require validation; however, they suggest that the epigenome may play a key role in the tolerance of patients to radiotherapy.

P-30

Searching a gene signature for risk of relapse prediction in patients with luminal A breast cancer.

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Breast cancer is one of the most common malignancies in women worldwide. Identifying prognostic biomarkers for predicting cancer progression is a significant problem due to its practical application in a clinical context for treating patients. This study aimed to identify the hub genes and to construct with then a prognostic signature that could predict the risk of relapse in patients with luminal A breast cancer.

We performed our objective in a cohort of mice with limited genetic heterogeneity previously generated in our laboratory by backcrossing (F1BX1 mice). Thus, we crossed MMTV-Neu transgenic mice in FVB genetic background with C57BL/6 mice resistant to breast cancer development. As a result of this crossing strategy, the F1BX1-neu mice are genetically unique. The free evolution of breast cancer was allowed in this mouse cohort and was characterized by evaluating a series of clinical pathophenotypes (latency, lifespan, number of tumors, metastasis, tumor growth rate, and tumor growth speed).

Expression arrays were done in tumors from F1BX mice. We identified differentially expressed genes (DEGs) between mouse groups with high and low Ncaph intratumoral expression levels, and network and enrichment analyses were conducted for this gene signature.

Hub genes of differentiated expression were identified by a multigene risk model constructed by multivariate LASSO regression,

The same hub genes were tested in a human database, and we obtained a risk model capable of predicting patient relapse similarly to PAM50 in patients with luminal A tumors.

P-31

The RNA methyltransferase METTL1 regulates cell migration in prostate cancer.

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CIC-CSIC, CIB-CSIC

Preliminary studies and data demonstrate a prominent and complex alteration of RNA-modifying proteins (RMPs) in prostate cancer (PCa), as is the case of the transfer RNA methyltransferase METTL1 (1), which is deregulated in advanced metastatic PCa. Very little is known about how RNA modifications can contribute to cancer progression, however recent studies have shown a correlation of this phenomenon with increased proliferation, self-renewal or migration (2-4).

In silico analysis showed that METTL1 was overexpressed in primary and metastatic tumours, which is correlated with poor prognosis. For functional characterization of METTL1 in the metastasis role, cells over-expressing METTL1 using TRIPZ-inducible vector or knocked-out for METTL1 (METTL1-KO) using CRISPR/Cas9 were generated in PCa cell lines. METTL1 deletion resulted in a reduction of cell migration and invasive growth in collagen matrices using in vitro approaches. We observed a decrease in

the junction of the actin fibres to the protein Phospho-Myosin Light Chain 2 (p-MLC2) in the METTL1-KO cells. Mechanistically, METTL1 deletion in cells led to a reduction of 7-methylguanosine (m7G) deposition in tRNAs, which favoured tRNA cleavage into tRNA-derived small non-coding RNA fragments, reprograming protein synthesis, increase stress response and increase ROS generation (5). ROS levels were rescued in the METTL1-KO cells by the re-expression of a wildtype form of METTL1, but not with a catalytic inactive METTL1. In addition, we rescued the migratory defect of METTL1-KO cells reducing ROS levels using ROS scavenger, as well as re-expression of a wildtype form of METTL1, but not with a catalytic inactive METTL1. Finally, we found that the migratory defect of METTL1-KO cells can be rescued blocking tRNA-derived small non-coding RNA fragments using antagomirs. Additionally, the new recent and ongoing results indicate a mitochondrial defect in the METTL1-KO cells. This could generate an energy disbalance, which explain the ROS accumulation and migratory defects in these cells.

Our study concludes that METTL1 regulates the migratory ability of the prostate cancer cells in vitro in a mechanism dependent of the tRNA fragments and ROS accumulation. Whether METTL1 implies a new possible therapeutic approach in PCa metastasis treatment needs further evaluation.

P-32

Uncover a lactate - sirtuin 6 crosstalk in the metabolic reprogramming of renal cell carcinoma.

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Introduction: Renal cell carcinomas (RCC), and especially clear cell RCC (ccRCC), the most lethal urological tumour, presents a poor prognosis in a metastatic form mostly due to the limited efficacy of available therapies. Recently, a metabolic-epigenetic interplay has been emerging in cancer, where metabolic fluctuations dictate cancer cells' epigenetic plasticity. Moreover, RCC display a characteristic Warburg effect, producing high levels of lactate oncometabolite. The effect of extracellular lactate on the epigenetic landscape, particularly in sirtuins (SIRTs) has been explored in some tumour types, however it remains uncharted territory in RCC.

Materials and Methods: The effects of lactate, nicotinamide (NAM) and alpha-cyano-4-hydroxycinnamate (CHC) on SIRT6 and histone acetylation levels were evaluated in normal kidney and RCC cell lines. Additionally, lactate derived SIRT6 effect on metabolic enzymes was assessed by qRT-PCR, western blot and Immunofluorescence. Finally, SIRT6 immunoexpression was tested in human RCC and normal renal tissues.

Results: Lactate decreased SIRT6 expression in RCC cells and normal kidney cells, increasing histone acetylation levels. This effect was paralleled by NAM treatment and reverted by lactate transporter inhibitor (CHC). Cells exposed to lactate exhibited increased PKM2, LDHA, MCT1 and MCT4 expression in RCC cells and normal kidney cells. Moreover, increased FASN and ACAC1 lipid synthesis-related genes was observed after lactate exposure in RCC cells. In addition, lactate increased the HIF-1α and HIF-2α transcript levels along with SREBP1 transcription factor. Importantly, primary tumors of RCC patients presented lower SIRT6 expression than normal kidney samples.

Conclusions: Lactate oncometabolite seems to regulate SIRT6 expression promoting a metabolic reprogramming in renal cell carcinoma cells.

P-33

N6-adenosine methyltransferase complex regulator ZC3H13 deletion as malignant prognosis factor in prostate cancer.

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Prostate Cancer (PCa) is the most common malignancy in men and the cause of highest mortality in developed countries. Despite improvement of diagnostic tools and therapies, more than 20% of patients still evolve to a more aggressive form, where treatment options are very limited revealing the need to find novel targeted molecular pathways that affect PCa.

By in silico screening using several datasets of genomic and expression data, CRISPR-ko and CRISPRa technology, pre-clinical models for metastatic PCa, and patients samples we aim to decipher the role of novel m6A regulators in PCa to implement novel therapeutic strategies.

Compelling evidence indicates that epitranscriptome plays a fundamental role in tumorigenesis. m6A deposition is catalysed by a methyltransferase complex formed by METTL3/METTL14 as the catalytic units and whose non-catalytic components are still emerging. Furthermore, m6A can be removed by FTO and ALKBH5 demethylases.

We identified that the epitranscriptomic regulator ZC3H13 is deleted and downregulated in 15% of PCa and 12% of metastasic Castration Resistant Prostate Cancer (mCRPC) patients, and it is associated with the recurrence, progression and poor prognosis. Gain- and loss-of-function analyses show that ZC3H13 regulates the proliferative, migratory and invasive capacities of PCa cells in vitro affecting cellular processes like adhesion or EMT. This phenotype can be rescued by pharmacologically inhibiting FTO demethylase. In addition, ZC3H13 overexpression reduces PCa cells metastatic potential in pre-clinical models. Mechanistically, we found that ZC3H13 stabilizes the core methylation complex in vitro and in patient samples, resulting in decreased m6A deposition levels upon ZC3H13 deletion.

In summary, we identify ZC3H13 as a prognostic factor in PCa. Our data further indicates that targeting m6A deposition levels in PCa inhibiting m6A mRNA demethylases alone or in combination with other therapeutic agents is a promising strategy to affect tumour growth and metastatic invasion.

P-34

Establishment of Patient-Derived Organoid (PDOs) cultures from breast cancer tumors: joys and sorrows.

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Tumor organoids are useful and robust culture systems of cancer cells derived from patients. Such ex-vivo three-dimensional tumor cultures display near-physiologic cellular composition and behaviors and can undergo extensive expansion in culture.

In order to study individual cancers and develop personalized treatments, our team has established PDO cultures derived from estrogen receptor (ER) positive tumors from breast cancer patients that participate in two clinical trials, NeoLetExe and NeoLetRib. Tumor samples have been collected at different time points: before, during and after treatment, and disassociated single cells have been seeded in 3D cell cultures. After optimization and adaptation of the PDO protocol published by Dekkers et al., 2021, more than 20 samples of PDOs have been biobanked reaching a success rate of higher than 85%.

Phenotypical characterization has shown differences between the samples. Phenotypes such as multicystic or dense organoids, irregular edges or regular rounded shapes are observed. Additionally, in order to characterize the cells that formed the tumor organoids, scRNA-seq has been performed on 6 samples. However, the analysis of scRNA-seq in the PDOs has demonstrated that the gene expression of the estrogen receptor 1 (ESR1) gene is dramatically reduced and other genes have changed their expression in comparison with the primary cells from the tumors.

Using different techniques and methods, we join forces with our collaborators in our European consortium (RESCUER, funded by EU Horizon project 2020) to try to identify what cells are in the PDOs and elucidate the mechanisms behind changes in the gene expression of cells in these PDOs. This will shed light on the understanding of ER expression regulation and can be of clinical relevance for ER-positive breast cancer treatments.

P-35

Identification of RNA modifications relevant for Docetaxel resistance in prostate cancer.

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Prostate cancer (PCa) is the most common malignancy in men in developed countries. While current therapies have shown survival benefits, many patients relapse and develop resistance, due to persistent cancer cells with the capacity to adapt to stress and self-renew. Thus, it is necessary to identify the molecular properties that determine the self-renewal and survival capacity of these cells, to develop efficient therapeutic strategies that target them. Based on the emerging evidence that implicates a key role of epitranscriptomic regulators in adaptation to stress signals, we propose to define the role of RNA modifications in therapy tolerance.

In order to find epitranscriptomic signatures that enhance cell survival two different approaches were addressed. A functional screening using the recently developed CRISPRa technology that allows to enhance the expression of any given gene using sgRNA that target TSS upstream sequences. To this aim we used a library containing more than 55,000 sgRNA guides targeting over 18,000 genes. After Docetaxel (DOC) treatment, enriched sgRNAs in treated cells were sequenced. In a second screening, we generated DOC-resistant PCa cell lines by exposure to increasing concentrations of DOC. Differential expression of epitranscriptomic regulators in DOC-resistant cells was analyzed. Finally, expression of candidate epitranscriptomic regulators was further validated in expression datasets from primary and castration-resistant metastatic PCa.

Our functional screenings have revealed that the expression of several epitranscriptomic regulator is significantly altered, such as the Elongator Complex subunits and the mitochondrial tRNA-modifying enzymes. More strikingly, we found that these tRNA modifying enzymes mainly catalyze positions in the anticodon and T-loop.

In summary, we have found that alterations of the expression of tRNA modifying enzymes lead to drug resistance in prostate cancer cells, thus suggesting that dynamic RNA modifications deposition onto mitochondrial and cytosolic tRNA regions may confer therapy tolerance to cancer cells. Further research is needed to elucidate the underlying molecular mechanism leading to the cancer cell survival associated phenotype and to validate future clinical implications of targeting these epitranscriptomic pathways.

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Pituitary neuroendocrine tumors subtypes exhibit specific methylome and transcriptome signatures.

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Pituitary adenomas are one of the most common benign neoplasms of the central nervous system, representing up to 15% of all primary brain tumors. As a minority of them may present aggressive behavior, the nomenclature Pituitary Neuroendocrine Tumors (PitNETs) was recently adopted. We aimed to explore PitNETs pangenomic signatures in a heterogeneous Brazilian ethnic population. Retrospective cross-sectional study Clinicopathological features, methylome, transcriptome and exome were evaluated in 77 patients (61% women, age: 12-72 years) followed due to functioning (FPT: GH-secreting n=18, ACTH-secreting n=14) and non-functioning pituitary tumors (NFPT, n=45), this study was approved by the Ethics Committee (#7534/2010) at HC-FMRP-USP. Unsupervised hierarchical clustering analysis (UHCA) of DNA methylation and transcription data revealed 3 PitNETs clusters each: (A) one enriched by FPT, (B) other by NFPT, and (C) another by ACTH-secreting and NFPT. Comparison between each omics-derived clusters identified 3,568 and 5,994 differentially methylated and expressed genes, respectively, associated with clinical presentation and invasiveness. UHCA considering 11 transcripts related to PitNETs 2022 WHO classification also supported the 3 clusters: POU1F1-driven somatotroph, TBX19-driven corticotroph, and NR5A1-driven gonadotroph PitNETs. Finally, we identified the set of genes and related pathways, supported by omics profile, that define each of the PitNETs elucidated clusters. This large Brazilian cohort of PitNETs confirms that, independently of the genetic background, integrated methylome and transcriptome signatures are capable to classify PitNETs, and are associated with clinical presentation and tumor invasiveness. Moreover, a third cluster (NFPT, USP8 mutated ACTH-secreting and silent-corticotroph PitNETs) raises interest regarding PitNETs heterogeneity. Finally, this data offers the opportunity to further studies to validate genes and pathways involved in the PitNETs pathogenesis.

P-37

Mapping RNA modifications in long-lived and cancer-resistant naked mole-rat

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Text submitted by Mikaela Behm.

P-38

Survival Meta-GWAS of recurrence after radiotherapy in NSCLC patients.

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Abstract

Objectives and purpose

To identify inherited genetic variants associated with the risk of time to recurrence after radiotherapy in non-small cell lung cancer (NSCLC) patients.

Material and methods

One hundred and sixty Galician patients from the RADIOGEN-Lung cohort and 167 Andalusian patients from the Hospital Universitario Vírgen del Rocío of Sevilla (HUVR) were included. Patients from the RADIOGEN-Lung cohort were prospectively recruited at the Radiation Oncology Department of the Hospital Clínico Universitario de Santiago de Compostela between March 2008 and May 2015, while Andalusian patients were recruited at the Radiation Oncology Department of the HUVR between 2013 and 2021. DNA was obtained from blood samples, which were then genome-wide genotyped and imputed. A cox proportional hazard-model was conducted to identify single-nucleotide polymorphisms associated with local and distant recurrence after radiotherapy. Covariates were selected by stepwise regression analysis between the clinical/pathological/dosimetry variables and the phenotype studied. A meta-analysis was performed with METAL, using p-value, direction of effect and sample size to study the SNP association.

Results

In local recurrence, we have identified a strong signal close to the TLL1 gene (strongest association rs72699109, meta-P-value: 7.03x10-08), which is related to the TFGB1 gene, previously described associated with radiotherapy toxicity in lung cancer patients. In distal recurrence, a signal in a non-coding region (strongest association rs7150684, meta-P-value: 1.46x10-06) has been identified.

Conclusions

Our meta-analysis identified a genetic signal associated with local recurrence in lung cancer patients after radiotherapy. We will validate this result in independent international cohorts.

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Potential role of HAKAI in N6-methyladenosine (m6A) RNA modification in colon cancer cells.

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mRNA methylation is one of the most common internal RNA modifications in eukaryotes and has emerged as a widespread regulatory mechanism that controls gene expression in various physiological and pathological processes. N6-methyladenosine (m6A) RNA modification, the most abundant modification on mRNA, plays an important role in cancer. m6A is installed by m6A methyltransferases (termed "writers"), removed by demethylases (termed "erasers"), and recognized by m6A-binding proteins (termed "readers"). Recently, it was reported that HAKAI, an E3 ubiquitin-ligase for E-cadherin, interacts with m6A writer components in Arabidopsis thaliana and Droshophila melanogaster, however, up to now, the role of HAKAI in m6A mRNA methylation in mammals is not fully understood. In this work, we aim to determine the possible role of HAKAI in colon cancer cells through its regulation in m6A. RNA immunoprecipitation (RIP) of m6A has been performed in HAKAI silencing HT29 cells compared to control HT29 cells, by using an inducible lentivirus system. RNA samples are extracted with trizol and reverse transcription was subsequently performed with the SuperScript VILO dDNA Synthesis kit. A cDNA library was constructed and the human gene expression profile of the transcriptome was analysed by Ion AmpliSeg™ Ion and Torrent 5S/XL sequencer. Bioinformatic data analysis was carried out to elucidate the differentially expressed genes. Our work will help us to add functional and molecular insights into the mechanism of HAKAI and its implication in m6A regulation in colon cancer cells.

P-40

GSTP1 as a potential biomarker for prostate cancer.

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Prostate cancer is a widespread disease that currently has no accurate diagnostic markers. Its early identification is important for the prevention of metastatic progression as well as treatment in its initial stages. The inability of the current PSA biomarker to distinguish between benign prostatic hyperplasia and prostate cancer has led to overdiagnosis and unnecessary biopsies, fuelling research to discover novel biomarkers. Our study considers the prospect of using the GSTP1 gene as a marker to differentiate between carcinomas and hyperplastic states. In our study, we analyzed biopsies of 20 patients with BPH and 20 biopsies of patients with PCa, assessing GSTP1 hypermethylation events at five CpG sites. We discovered statistically significant differences in methylation patterns between BPH and PCa and were able to positively correlate methylation to tPSA and age. Furthermore, PCa CpG islands were consistently more hypermethylated than BPH tissues, and interestingly, PCa tumour-adjacent tissue (TAT) showed a similar progression towards hypermethylation states compared to BPH. These results were also confirmed when assessing the GSTP1 protein expression, which was absent in PCa, but was present in BPH and TAT. Concerning our results, we feel that this marker would be an excellent candidate to enhance current diagnostic tools in PCa identification.

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MAPKAPK5AS1 lncRNA regulates the growth of T-cells by an m6A mediated mechanism.

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MAPKAPK5-AS1 is an uncharacterized lncRNA that has been associated with the development of different types of cancer. This lncRNA carries a SNP (rs3177647) that is closely located to an m^{6}A methylation motif, and has been previously described as m^{6}A-eQTL. Additionally, this SNP is associated with lymphocyte count and with the development of different autoimmune disorders, which has been related to increase risk to develop certain types of cancer. Therefore, we hypothesized that SNP-related methylation changes could affect MAPKAPK5-AS1 expression and function, influencing lymphocyte count and contributing to the development of blood cancers.

To test our hypothesis, we used the Jurkat T-cell line heterozygous for the m6A-QTL SNP rs4346023. m6A-immunoprecipitation assay in Jurkat cells confirmed MAPKAPK5-AS1 allele specific methylation, with the (T) allele associated with higher T cell count presenting lower methylation levels. Additionally, manipulation of the m6A machinery by silencing the eraser ALKBH5, revealed a decrease in the expression levels of the lncRNA, confirming its m6A-mediate regulation. Silencing of MAPKAPK5-AS1 resulted in an increase of IFNG and a decrease of IL2, suggesting that reduced levels of this lncRNA generate a protective scenario for the development of blood cancers. Mutant cells for MAPKAPK5-AS1 and for the m6A motif generated by CRISPR-Cas9 also showed higher levels of IFNG. Moreover, we confirmed that cells with lower MAPKAPK5-AS1 expression presented reduced growth rate, supporting the possible implication of this lncRNA in the regulation of T-cell growth. Analysis of online available malignant hematopoiesis data using BloodSpot database, confirmed that Acute myeloid leukemia patients with genetic aberrations generally have increased levels of MAPKAPK5-AS1 and IL-2 and lower IFNG levels.

In brief, our observations, shed light on how a noncoding m^{6}A-QTL can influence lymphocyte count, mediating predisposition to develop blood cancer. Our results open the door to novel therapeutic approaches for anti-tumor immune response based on lncRNA and m6A regulation.

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Cell subtype identification using marker-free light microscopy and AI.

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The discrimination between cell types and/or states is an important step in areas like cancer heterogeneity or stem cell differentiation, among others. There are recent powerful tools for recognizing cell types that rely on single-cell RNA-seq or spatial transcriptomics but they are difficult to translate into in vitro experiments. In order to solve this, imaging techniques are being developed to identify specific cell types, but they usually depend on cell markers and good optical conditions. Here, we present an innovative method that is able to identify different cell types and even between cellular states in multiple conditions. We show that subcellular regions are powerful enough to classify cell types using a deep learning neural network that is trained with a set of tens of images obtained by light microscopy per type. This marker-independent model is able to discriminate between distinct cell types, and is useful for the relative quantification of cell types within either a differentiation or a wound-healing experiment. In addition, we have found that our new method is subtle enough to discriminate between cell states, as the number of passages in a stem cell model. In summary, we present a general tool to discriminate cellular identities that can be easily adapted to multiple functions and experiments in cancer.

P-43

Patterns of differentially expressed circRNAs in human thymocytes.

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Circular RNAs (circRNAs) are suggested to play a discriminative role between some stages of thymocyte differentiation. However, differential aspects of the stage of mature single-positive thymocytes remain to be explored. The purpose of this study is to investigate the differential expression pattern of circRNAs in three different development stages of human thymocytes, including mature single-positive cells, and perform predictions in silico regarding the ability of specific circRNAs when controlling the expression of genes involved in thymocyte differentiation. We isolate human thymocytes at three different stages of intrathymic differentiation and determine the expression of circRNAs and mRNA by RNASeq. We show that the differential expression pattern of 50 specific circRNAs serves to discriminate between the three human thymocyte populations. Our study provides, for the first time, significant insights into the usefulness of circRNAs in discriminating between different stages of thymocyte differentiation and provides new potential circRNA-miRNA-mRNA networks capable of controlling the expression of genes involved in T-cell differentiation in the thymus.

P-44

Compression of resistance mechanisms to treatment to kinases cyclin-dependent CDK4/6 by determining changes in chromatin in patients with advanced breast cancer.

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CIC.

It has been reported in clinics that luminal B subtype (hormone receptor+ and HER2-) treated with cyclin-dependent kinases (CDK) 4/6 inhibitors in combination with aromatase inhibitors (AI) as a neoadjuvant therapy upregulates HER2 expression. However, the chromatin landscape alteration created by CDK4/6 combined with ER inhibition in breast cancer has not been explored yet. In this study, we have demonstrated that CDK4 kinase phosphorylates FOXA1 at serine 234, 307 and 331 residues and that CDK4/6 inhibitors reprogram binding of transcription factor FOXA1. This chromatin accessibility reprograming mediated by FOXA1 results in an increase in the HER2 signaling pathway.

P-45

Epigenetic characterization of partial-EMT state in Oral Squamous Cell Carcinomas.

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Oral squamous cell carcinomas (OSCCs) represent one of the most common head and neck cancers (HNCs), which are mostly caused by tobacco and alcohol exposure. These have high risk of recurrence and metastasis, and low survival rate. Consequently, there is an actual need for understanding the molecular processes underlying OSCC progression. Recent scRNAseq analysis on HNCs discovered a discrete population of cancer cells expressing a signature of partial epithelial to mesenchymal transition (pEMT)1, which was correlated with the presence of metastatic disease. In cutaneous-SCC, which are related to HNCs, pEMT state is defined at the transcriptional level by the co-expression of epithelial (TP63/SOX2) and mesenchymal (SNAI1/2, ZEB1/2) transcription factors (TFs)2. Interestingly, HNCs pEMT positive cells failed to co-express mesenchymal TFs1. This result could be explained by the existence of alternative mechanisms that induce pEMT programs, or to an artifact caused by the low sensitivity of the scRNAseq technology.

To understand how pEMT programs are regulated in OSCCs, we have purified pEMT low and high tumor cells from OSCCs patient biopsies to perform bulk RNAseq and characterized potential signaling pathways and TFs controlling their function. In parallel, we have profiled their chromatin accessibility and histone modifications to identify TF binding and enhancer activities regulating pEMT cells. Interestingly, our data suggest that pEMT phenotype may be controlled by two EMT TFs and that it requires the downregulation of the epithelial TF SOX2 but not TP63. In addition, some epigenetic remodelers that are specifically expressed in pEMT cells may contribute to modify the chromatin state to allow the epithelial to partial mesenchymal transition.

To analyze the function of EMT/epithelial TFs in pEMT regulation, we look for our established pEMT signature in OSCC cell lines from CCLE collection, uncovering that pEMT programs are not fully conserved in commercial cells lines grown in 2D conditions. Conversely, we identified that 3D tumoroids models developed in our laboratory from OSCC patient biopsies, recapitulate transcriptionally and epigenetically pEMT phenotypes. These models will serve as a promising platform to study pEMT function and how we can interfere it to prevent metastatic spread.

P-46

Beyond Neurosurgical Limits (Part I): A Novel Brain Organoid 3D-culture Model for Glioblastoma Local Therapy Simulation.

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Glioblastoma multiforme (GBM) has a dramatically quick progression and poor outcomes even under the best surgical and medical treatment. Investigation of novel therapies calls for a model that can realistically emulate the paradigmatic progression and the natural interaction between GBM and surrounding brain tissue. Here we propose a human iPSC-derived cerebral organoid as an experimental model for studying GBM in vitro, allowing direct observation of tumor initiation, infiltration and interaction within the brain microenvironment. The human iPSC-derived cell line BJ-iPS-SV4F-9 grew into self-organized brain-like structures (organoids). When these organoids were co-cultured with glioblastoma initiating cells pre-labeled with GFP (GFP-GICs), they allowed for efficient tumor engraftment, beginning within 24 hours. Between days 15-40, both cell subtypes, the proneural GFP-GIC7 and the mesenchymal GFP-PG88, invaded and extensively grew into the organoid. GFP-PG88 engraftment was equally successful either in the form of tumorspheres or as single cells; in contrast, GFP-GIC7 seeded as single cells were more efficient than seeded as tumorspheres. The presented model emulated the natural interaction between glioblastoma and glial cells; particularly, when GFAP-positive astrocytic cells were seen forming a reactive scar around the tumor on day 31 of the co-culture. In summary, brain organoids co-cultured with GFP-GICs are a powerful system to study the pathogenesis of GBM. This symbiosis provides a realistic insight of the tumor-microenvironment interaction and might become a useful tool to test local therapies targeting the postsurgical resection cavity.

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