cnio "LA CAIXA" FRONTIERS MEETINGS 2019

Madrid 23 — 25 Sept 2019

Heterogeneity and Evolution in Cancer

Organisers

Fátima Al-Shahrour
Spanish National Cancer Research Centre, CNIO, Madrid, Spain

Arnold Levine
The Simons Center for Systems Biology, Institute for Advanced Study, Princeton, US

Solip Park
Spanish National Cancer Research Centre, CNIO, Madrid, Spain

Raúl Rabadán
Columbia Systems Biology, Columbia University, New York, US

Speakers

Alexander R.A. Anderson
Moffitt Cancer Center and Research Institute, Tampa, US

Niko Beerenwinkel
ETH Zürich, Switzerland

Ivana Bozic
University of Washington, US

Curtis G. Callan
Princeton University, US

Neal G. Copeland
University of Texas MD Anderson Cancer Center, Houston, US

Christina Curtis
Stanford University, School of Medicine, Stanford, US

Adolfo Ferrando
Institute for Cancer Genetics, Columbia University Medical Center, New York, US

Trevor Graham
Barts Cancer Institute, London, UK

Benjamin D. Greenbaum
Icahn School of Medicine at Mount Sinai, New York, US

Holger Heyn
National Centre for Genomic Analysis, Barcelona, Spain

Nancy Jenkins
University of Texas MD Anderson Cancer Center, Houston, US

Tal Korem
Columbia University Irving Medical Center, New York, US

Christina Leslie
Memorial Sloan Kettering Cancer Center, New York, US

Arnold Levine
The Simons Center for Systems Biology, Institute for Advanced Study, Princeton, US

Nuria Lopez-Bigas
Institute for Research in Biomedicine, Barcelona, Spain

Scott W. Lowe
Memorial Sloan Kettering Cancer Center, New York, US

Guillermina Lozano
Univerity of Texas Anderson Cancer Centre, Houston, US

Marta Łuksza
Icahn School of Medicine, Mount Sinai, New York, US

David Posada
School of Biology, University of Vigo, Spain

Carol Prives
Columbia University, New York, US

Benjamin J. Raphael
Lewis-Sigler Institute for Integrative Genomics, Princeton University, US

Darryl Shibata
Keck School of Medicine of USC, Los Angeles, US

Andrea Sottoriva
The Institute of Cancer Research, London, UK

Doug Winton
Cancer Research UK Cambridge Institute, Cambridge, UK

EXCELENCIA SEVERO OCHOA 2012 - 2019
GOBIERNO DE ESPAÑA · MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES · Instituto de Salud Carlos III
"la Caixa" · cnio stop cancer

Madrid 23 — 25 Sept 2019

Heterogeneity and Evolution in Cancer

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Heterogeneity and Evolution in Cancer

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cnio "LA CAIXA" FRONTIERS MEETINGS 2019

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Heterogeneity and Evolution in Cancer

Summary

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Madrid 23 — 25 Sept 2019

Heterogeneity and Evolution in Cancer

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cnio "LA CAIXA" FRONTIERS MEETINGS 2019

Madrid 23 — 25 Sept 2019

Heterogeneity and Evolution in Cancer

Organisers and Speakers

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Madrid 23 — 25 Sept 2019

Heterogeneity and Evolution in Cancer

Venue:

Spanish National Cancer Research Centre – CNIO Auditorium, Madrid, Spain

Chairpersons and organising committee:

Fátima Al-Shahrour
Spanish National Cancer Research Centre, CNIO, Madrid, Spain

Arnold Levine
The Simons Center for Systems Biology, Institute for Advanced Study, Princeton, US

Solip Park
Spanish National Cancer Research Centre, CNIO, Madrid, Spain

Raúl Rabadán
Columbia Systems Biology, Columbia University, New York, US

Rationale:

Cancers are dynamical and heterogeneous biological processes. Heterogeneity and evolution are the main factors driving oncogenesis, growth, invasion and failure of therapy. Longitudinal, multi-sampling and single cell genomic and transcriptomic characterization of tumors allows to reconstruct their changes and to link them to clinically relevant phenotypes. This workshop will bring together a community of interdisciplinary researchers developing tools to study these processes and applying them to large numbers of tumors.

Speakers

Alexander R.A. Anderson
Moffitt Cancer Center and Research Institute, Tampa, US

Niko Beerenwinkel
ETH Zürich, Switzerland

Ivana Bozic
University of Washington, US

Curtis G. Callan
Princeton University, US

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cnio "LA CAIXA" FRONTIERS MEETINGS 2019

Madrid 23 — 25 Sept 2019

Heterogeneity and Evolution in Cancer

Programme

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23rd September 2019 - Monday

09:00 *Registration*

09:30 - 09:45 Opening Remarks
(Fátima Al-Shahrour and Raul Rabadan)

09:45 - 12:45 S#1
Chairperson: Raul Rabadan

09:45 - 10:15 *"The roles of initiating truncal mutations in human cancers: the order of mutations and the tumor cell type matters"*
Arnold Levine
The Simons Center for Systems Biology, Institute for Advanced Study, US

10:15 - 10:45 *"Rapid evolution and biogeographic spread in a colorectal cancer"*
David Posada
School of Biology, University of Vigo, Spain

10:45 - 11:15 Group picture (main door) & coffee break (social room)

11:15 - 11:45 *"Single-Cell RNA sequencing: from sample to cell atlas"*
Holger Heyn
National Centre for Genomic Analysis (CNAG-CRG), Barcelona, Spain

11:45 - 12:00 SHORT TALK *"Tracing the evolutionary history of clonal competition in mouse polymutated esophageal epithelium"*
Gabriel Piedrafita
Spanish National Cancer Research Centre, Madrid, Spain

12:00 - 12:15 SHORT TALK *"Genomic and immunological study of response to anti-PD-1 immunotherapy in glioblastoma"*
Andrew Chen
Columbia University, New York, US

23rd September 2019 - Monday

12:15 - 12:45 *"Learning tumor phylogenies from single-cell data"*
Niko Beerenwinkel
ETH Zürich, Switzerland

12:45 - 14:15 Lunch break (cafeteria)

14:15 - 17:00 S#2
Chairperson: Arnold Levine

14:15 - 14:45 *"Harnessing transposon mutagenesis to study tumor evolution and tumor heterogeneity"*
Neal G. Copeland
University of Texas MD Anderson Cancer Center, US

14:45 - 15:00 SHORT TALK *"Cell-of-origin of prostate cancer and clinical heterogeneity"*
Esther Baena
CRUK Manchester Institute, UK

15:00 - 15:15 SHORT TALK *"A clonal expression biomarker improves prognostic accuracy in lung cancer"*
Dhruvas Biswas
UCL Cancer Institute, London, UK

15:15 - 15:45 *"Transposon mutagenesis identifies genes and evolutionary forces driving gastrointestinal tract tumor progression"*
Nancy Jenkins
University of Texas MD Anderson Cancer Center, US

15:45 - 16:15 Coffee break (social room)

16:15 - 16:30 SHORT TALK *"MGMT genomic rearrangements contribute to chemotherapy resistance and tumour relapse in gliomas"*
Massimo Squatrito
Spanish National Cancer Research Centre, Madrid, Spain

23rd September 2019 - Monday

16:30 - 17:00 *"Cancer drivers and dependencies"*
Scott W. Lowe
Memorial Sloan Kettering Cancer Center, New York, US

17:00 - 18:00 Poster Session & Wine and cheese (social room)

24th September 2019 - Tuesday

09:00 - 11:15 S#3
Chairperson: Scott Lowe

09:00 - 09:30 *"The statistical ensemble approach to adaptive immunity"*
Curtis Callan
Physics Department, Princeton University, Princeton, US

09:30 - 10:00 *"Mutant p53 activities in somatic models of osteosarcoma and breast cancer"*
Guillermina Lozano
University of Texas Anderson Cancer Center, US

10:00 - 10:15 SHORT TALK *"Predicting tumor evolution using cancer progression models"*
Ramón Díaz Uriarte
Autonomous University of Madrid, Spain

10:15 - 10:45 *"Quantifying tumor evolution across time and space*
Benjamin J. Raphael
Lewis-Sigler Institute for Integrative Genomics, Princeton, US

10:45 - 11:15 *"Heterogeneous Immune Determinants of Tumor Evolution"*
Benjamin D. Greenbaum
Icahn School of Medicine at Mount Sinai, New York, US

11:15 - 11:45 Coffee break (social room)

24th September 2019 - Tuesday

11:45 - 13:30 **S#4**

Chairperson: Fátima Al-Shahrour

11:15 - 11:45 *"Age related mutational burden in human colonic epithelium is dictated by stem cell renewal processes"*
Douglas J. Winton
Cancer Research UK Cambridge Institute, Cambridge, UK

11:45 - 12:15 *"Tumor genomes shed light into somatic mutational processes and cancer vulnerabilities"*
Nuria Lopez-Bigas
Institute for Research in Biomedicine, Barcelona, Spain

12:15 - 12:30 SHORT TALK *"Intratumour heterogeneity in a pancreatic cancer mouse model"*
Laura González Silva
IBBTEC, Santander, Spain

12:30 - 13:00 *"Clonal evolution mechanisms in leukemia initiation and relapse"*
Adolfo Ferrando
Institute for Cancer Genetics, Columbia University, NY, US

13:00 - 13:30 *"Measurement of selective coefficients for subclonal mutations in human cancer"*
Trevor Graham
Barts Cancer Institute, London, UK

13:30 - 15:00 Lunch break (cafeteria)

24th September 2019 - Tuesday

15:00 - 17:00 **S#5**

Chairperson: Solip Park

15:00 - 15:30 *"Complex roles of p53, Mdm2 and MdmX in survival and death of cancer cells"*
Carol Prives
Columbia University, New York, US

15:30 - 16:00 *"Organizing spatial and temporal intratumoral heterogeneity on microscopic tissue sections"*
Darryl Shibata
Keck School of Medicine of USC, Los Angeles, US

16:00 - 16:15 SHORT TALK *"Inference of mutational heterogeneity and variant clonality reveals distinct genomic landscape for hematopoietic cells admixed in solid tumor microenvironment"*
Hossein Khiabanian
Rutgers Cancer Institute of New Jersey, US

16:15 - 16:30 SHORT TALK *"Rational therapeutic combination with BH3 mimetics to overcome cancer adaptationto treatment"*
Joan Montero
Institute for Bioengineering of Catalonia, Barcelona, Spain

16:30 - 17:00 *"Delineating the rates and routes of metastasis"*
Christina Curtis
Stanford University, School of Medicine, Stanford, US

17:00 - 18:00 Posters Session & Snacks (social room)

25th September 2019 - Wednesday

09:30 - 13:30 **S#6**

Chairperson: Núria López-Bigas

09:30 - 10:00 *"Exploiting evolutionary steering to control drug resistance in cancer"*
Andrea Sottoriva
The Institute of Cancer Research, London, UK

10:00 - 10:30 *"Immune interactions predict cancer evolution"*
Marta Łuksza
Icahn School of Medicine, Mount Sinai, New York, US

10:30 - 10:45 SHORT TALK *"Characterizing transcriptional and genomic heterogeneity in circulating tumor cells"*
Francesc Castro
University of Basel, Basel, Switzerland

10:45 - 11:00 SHORT TALK *"Phenotypic heterogeneity (not genetic) underpins survival during induction chemotherapy in childhood acute lymphoblastic leukaemia"*
Virginia Turati
UCL Cancer Institute, London, UK

11:00 - 11:30 *"Epigenetic rewiring and tumor plasticity*
Christina Leslie
Memorial Sloan Kettering Cancer Center, New York, US

11:30 - 12:00 *Coffee break (social room)*
Certificate of Attendance and invoice will be available at the reception desk

25th September 2019 - Wednesday

12:00 - 12:30 *"Mathematical modeling of cancer heterogeneity"*
Ivana Bozic
University of Washington, Washington, US

12:30 - 13:00 *"Evolutionary therapy for metastatic cancer"*
Alexander R. A. Anderson
Moffitt Cancer Center & Research Institute, Tampa, US

13:00 - 13:30 *"Inferring microbial activity from metagenomic data"*
Tal Korem
Columbia University Irving Medical Center, New York, US

13:30 closing remarks, short talks & poster Awards

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Madrid 23 — 25 Sept 2019

Heterogeneity and Evolution in Cancer

23rd September 2019 — Monday

Session #1

Chairperson: ***Raul Rabadan***

The roles of initiating truncal mutations in human cancers: the order of mutations and the tumor cell type matters*

Arnold Levine
The Simons Center for Systems Biology,
Institute for Advanced Study, US

Evidence will be presented to demonstrate that initiating truncal mutations in cancers play a special role in expanding clones of specific cell types, enhancing their survival and selecting for a subset of specific additional mutations that go on to produce a malignant cancer. This preferential order of mutations rapidly transitions benign to malignant tumors. This idea helps to explain why inherited cancer predispositions produce tumors of tissue types different from spontaneous mutations in the same gene or the same allele. Initiating mutations in a tumor make excellent targets for immunotherapy, and understanding the path of natural selection of mutations in a tumor should enhance our ability to prevent benign tumors from becoming malignant tumors.

*Arnold Levine, Nancy Jenkins, and Neal Copeland

Rapid evolution and biogeographic spread in a colorectal cancer

David Posada
Center of Biomedical Research (CINBIO),
School of Biology, University of Vigo,
Vigo, Spain

How and when tumoral clones start spreading to surrounding and distant tissues is currently unclear. Here, we applied a sophisticated evolutionary framework to describe the evolutionary history of a colorectal cancer in time and space. In particular, we have leveraged state-of-the-art approaches from statistical phylogenetics, phylodynamics, and biogeography that allowed us to date the origin of the tumor, to quantify its demography, and to identify the different colonization events that took place. Thus, our analyses strongly support an early monoclonal metastatic colonization, followed by a rapid population expansion at both primary and secondary sites. Moreover, we infer a hematogenous metastatic spread seemingly under positive selection, plus the return of some tumoral cells from the liver back to the colon lymph nodes. This study provides with unprecedented detail a picture of the tempo and mode of the tumoral clonal dynamics within a single patient. Importantly, it exemplifies how sound methods from organismal evolutionary biology can be ported to the within-individual level in order to understand complex tumoral dynamics over time and space.

Single-Cell RNA sequencing: from sample to cell atlas

Holger Heyn[1,2]

[1]Centro Nacional de Análisis Genómico (CNAG-CRG) - Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain
[2]Universitat Pompeu Fabra (UPF), Barcelona, Spain

Single-cell RNA sequencing (scRNAseq) is at the forefront of techniques to chart molecular properties of individual cells. Recent methods are scalable to thousands of cells, enabling an unbiased sampling and in-depth characterization without prior knowledge. Consequently, studies aim to produce comprehensive cell atlases of healthy and diseased tissues and organs. However, there are large differences between scRNAseq techniques and it remains elusive which protocols are most adequate to draw a cell atlas. Therefore, we generated benchmark datasets to systematically evaluate techniques for their power to describe cell types and states comprehensively. The methods revealed large differences in their performance, which has to be considered when defining guidelines and standards in the framework of international consortia, such as the HCA project. Moreover, we performed a benchmarking study of sampling biases that could arise in large patient cohorts and biobank projects. Particularly, we tested the effect of sampling time on the gene expression profiles of peripheral blood mononuclear and leukemia cells. We detected a systematic bias introduced during sample processing, an important features that need to be taken into account when designing experiments for population-based association studies. Failing to select suitable samples or to correct datasets during the analysis can lead to biased or false reporting.

Tracing the evolutionary history of clonal competition in mouse polymutated esophageal epithelium

SHORT TALK

Gabriel Piedrafita[1,2], Bartomeu Colom[1], Philip H Jones[1]

[1]Wellcome Sanger Institute, Hinxton, Cambridge, UK.
[2]Present address: Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Somatic tissues accumulate a wide range of mutations as we age. By adulthood, normal human epithelia consist of a heterogeneous patchwork of mutant clones, many of which contain alterations in cancer-related driver genes. While these constitute a suitable pre-stage for tumorigenesis, little is known about the cellular processes and evolutionary constraints that shape this complex mutational landscape. In this work, we investigate this issue in the esophageal epithelium of adult mice following mutagen treatment. We combine deep DNA sequencing with genetic lineage tracing to get unprecedented spatio-temporal resolution on clonal competition dynamics during field cancerization. The esophageal epithelium developed a high burden of mutations, with signs of strong positive selection in several prevalently-mutated genes, including Notch1 and Trp53. Quantitative lineage tracing revealed accelerated clone loss and expansion of surviving clones over time, indicative of early strong clonal competition dynamics, which were though progressively tempered down towards neutrality. Despite the wide mutational diversity, global-scale clone dynamics were strikingly recapitulated by a simple, lattice-based model in which the competitive fitness of a mutant cell is a function of its differentiation propensity relative to neighbors. We conclude differentiation fate imbalance and spatial clonal competition at clashing borders are main forces contributing to the clonal landscape in poly-mutated normal epithelium.

SHORT TALK

Genomic and immunological study of response to anti-PD-1 immunotherapy in glioblastoma

Junfei Zhao, **Andrew X. Chen,** Robyn D. Gartrell, Andrew M. Silverman, Luis Aparicio, Tim Chu, Darius Bordbar, David Shan, Jorge Samanamud, Aayushi Mahajan, Ioan Filip, Rose Orenbuch, Morgan Goetz, Jonathan T. Yamaguchi, Michael Cloney, Craig Horbinski, Rimas V. Lukas, Jeffrey Raizer, Ali I Rae, Jinzhou Yuan, Peter Canoll, Jeffrey N. Bruce, Yvonne M. Saenger, Peter Sims, Fabio M. Iwamoto, Adam M. Sonabend and Raul Rabadan

[1]Departments of Systems Biology, Biomedical Informatics, Pediatrics, Pediatric Hematology/Oncology/SCT, Neurosurgery, Pathology and Cell Biology, Medicine, Hematology/Oncology, Neurology at Columbia University, US
[2]Department of Biomedical Engineering at University of North Carolina at Chapel Hill, US
[3]Departments of Neurological Surgery, Pathology, Neurology at Northwestern University Feinberg School of Medicine, US
[4]Department of Neurological Surgery at Oregon Health & Sciences University, US

Immune checkpoint inhibitors have been successful against many cancers; however, their efficacy has been uncommon and unpredictable in glioblastomas (GBM), where <10% of patients show long-term responses. To understand the molecular determinants of immunotherapeutic response in GBM, we have longitudinally profiled 66 patients, including 17 long-term responders, during standard therapy and after treatment with PD-1 inhibitors. We found a significant enrichment of PTEN mutations associated with immunosuppressive expression signatures in non-responders, and an enrichment of MAPK pathway alterations (PTPN11, BRAF) in responders. Responsive tumors were also associated with branched patterns of evolution from the elimination of neoepitopes, as well as differences in T cell clonal diversity and tumor microenvironment profiles. We show that clinical response to anti-PD-1 immunotherapy in GBM is associated with specific molecular alterations, immune expression signatures, and immune infiltration that reflect the tumor's clonal evolution during treatment.

Learning tumor phylogenies from single-cell data

Niko Beerenwinkel
ETH Zürich,
Zürich, Switzerland

Cancer progression is an evolutionary process characterized by the accumulation of mutations and responsible for tumor growth, clinical progression, and drug resistance development. We discuss how to reconstruct the evolutionary history of a tumor from single-cell sequencing data. The tumor phylogeny problem is challenging because of sequencing errors and the high rate of allelic drop-out in single-cell DNA sequencing experiments. We present probabilistic models and efficient inference algorithms for mutation calling and learning tumor phylogenies from such data.

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Heterogeneity and Evolution in Cancer

23rd September 2019 — Monday

Session #2

Chairperson: ***Arnold Levine***

Harnessing transposon mutagenesis to study tumor evolution and tumor heterogeneity

Neal G. Copeland
Professor of Practice
University of Texas, MD Anderson Cancer Center,
Houston, Texas, US

Sleeping Beauty (SB) transposon-based insertional mutagenesis provides a powerful tool for cancer gene discovery in both hematopoietic and solid tumors. SB can identify initiating truncal and progression drivers as well as potential metastatic drivers at a level not achievable in human tumors with current sequencing technology. It can also pinpoint cancer drivers that are difficult to find with other approaches, such as genes that are deregulated by mutations in regulatory sequences or through epigenetic means, and thus complements the sequencing-based census of human cancer genes. Recently, we and others have described methods for semiquantitative transposon insertion site sequencing (QiSeq) that exploits acoustic fragmentation of tumor DNA to reduce bias inherent in widely used restriction-digestion-based approaches for cloning and sequencing SB insertion sites. In addition, we have described a new transposon-based, liquid-phase, capture sequencing and bioinformatics pipeline (SBCapSeq) that makes it possible to sequence transposon insertion sites from single tumor cells. When applied to cancer gene discovery in SB-driven mouse models of undifferentiated leukemia, pancreatic cancer and melanoma, SBCapSeq made it possible to unambiguously identify genes and signaling pathways driving these diseases. Trunk drivers could now be identified with confidence, as could both interacting and non-interacting trunk drivers. SB transposition can be activated in virtually any cell type, with or without a pre-existing(s) cancer mutation, to model almost any type of cancer. SBCapSeq therefore offers an unprecedented method for studying tumor evolution at a single cell resolution in potentially any SB-driven disease.

Cell-of-origin of prostate cancer and clinical heterogeneity

SHORT TALK

Esther Baena

CRUK Manchester Institute,
Manchester, UK

Personalized treatment for prostate cancer remains a challenge because no clear molecular subtypes allow for risk stratification and prediction of response to treatment. Imaging, PSA levels and pathological assessment of biopsies through the Gleason grading system remain the gold standard for diagnosis and risk stratification. Moreover, most genomic campaigns analysed single biopsies with limited analysis of their cellular landscape, not considering that prostate cancer is a multifocal disease. By combining genomic and multiparametric imaging analysis of high-risk prostate cancer patients, we have characterized the radiogenomic landscape of multifocal prostate cancer (Parry, Srivastana, Ali et al, EU Oncology 2019). Moreover, coupling single-cell profiling and functional characterization by organoid-culture and in situ lineage-tracing analysis in mouse models, we have identified inherently castration-resistant cellular subpopulations in the prostate defined by their unique cell-surface markers. In particular, our studies define LY6D as a marker for prostate progenitors and castration-resistant luminal cells, which may serve as prognostic maker for advanced prostate cancer (Barros-Silva, Linn, Steiner, Cell Reports 2018). Further functional and longitudinal characterisation of the identified therapy-resistant prostate luminal subpopulations highlights their contribution to tumour subtypes, thereby advancing patient stratification and setting a pipeline to develop novel therapeutics.

SHORT TALK

A clonal expression biomarker improves prognostic accuracy in lung cancer

Dhruva Biswas[*1,2,3], Nicolai J Birkbak[*#1,3,4,5], Rachel Rosenthal[1,2,3], Crispin T. Hiley[1,3], Emilia L. Lim[1,3], Krisztian Papp[6], Stefan Boeing[7], Marcin Krzystanek[8], Dijana Djureinovic[9], Linnea La Fleur[9], Maria Greco[9], Balázs Döme[11,12,13], János Fillinger[14,15], Hans Brunnström[16], Yin Wu[1], David A. Moore[18], Marcin Skrzypski[1,19], Christopher Abbosh[1], Kevin Litchfield[3], Maise Al Bakir[3], Thomas BK Watkins[3], Selvaraju Veeriah[1], Gareth A. Wilson[1,3], Mariam Jamal-Hanjani[1], Judit Moldvay[11,17], Johan Botling[9], Arul M. Chinnaiyan[20,21,22,23,24], Patrick Micke[9], Allan Hackshaw[25], Jiri Bartek[8,26], Istvan Csabai[6], Zoltan Szallasi[8,17,27], Javier Herrero[2], Nicholas McGranahan[#1,28], and Charles Swanton[#1,3], on behalf of the TRACERx consortium.

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At the point of cancer diagnosis, molecular biomarkers aim to stratify patients into disease subtypes predictive of outcome, providing precision beyond standard clinical descriptors such as tumour stage. Transcriptomic intra-tumour heterogeneity (RNA-ITH) has been shown to confound existing molecular biomarkers across multiple cancer types. Here, we analyse multi-region wholeexome and RNA sequencing data for 156 tumour regions from 48 TRACERx patients to explore and control for RNA-ITH in non-small cell lung cancer (NSCLC). We find that chromosomal instability (CIN) is a major driver of RNAITH, and existing prognostic gene expression signatures are vulnerable to tumour sampling bias. To address this, we identify genes expressed homogeneously within individual tumours that are often driven by clonal DNA copy-number gains selected early in tumour evolution and encode expression modules of cancer cell proliferation. Clonal transcriptomic biomarkers overcome tumour sampling bias, improve prognostic accuracy over current clinicopathological risk factors, and may have general applicability as a strategy to refine biomarker design across cancer types.

Transposon mutagenesis identifies genes and evolutionary forces driving gastrointestinal tract tumor progression

Nancy A. Jenkins

Professor of Practice
University of Texas, MD Anderson Cancer Center,
Houston, Texas, US

To provide a more comprehensive understanding of the genes and evolutionary forces driving colorectal cancer (CRC) progression, we performed transposon mutagenesis screens in mice carrying mutations in genes that act at different stages of human CRC progression. This allowed us to identify a set of genes that appear highly relevant for CRC and provide a better understanding of the evolutionary forces and systems properties of CRC. These studies also showed that the order of addition of mutations driving CRC matters, and that these mutations act in a tissue-preferred fashion. I will also describe the engineering of an organotypic colon cancer model we developed through the recellularization of native human colon matrix that recapitulates the pathophysiological progression from APC-mutant neoplasia to submucosal invasive tumor, which made it possible to identify candidate genes that drive the inititial stages of tumor metastases. Finally, I will describe a platform for functionally validating CRC driver genes identified in mouse transposon screens and human CRC sequencing studies that utilizes CRISPR-Cas9 in mouse intestinal tumor organoids and human CRC-derived organoids in xenograft mouse models. These studies made use of genetically defined benign tumor-derived organoids carrying two frequent mutations (APC and KRAS) that act in the early stage of CRC development, so that the tumorigenic ability of a mutation in a single gene could be clearly evaluated. This experimental system can also be applied to mouse intestinal organoids carrying other sensitizing mutations as well as organoids derived from other organs, which could further contribute to the identification of novel cancer driver genes and new drug targets.

SHORT TALK

MGMT genomic rearrangements contribute to chemotherapy resistance and tumour relapse in gliomas

Oldrini Barbara[1], Nuria Vaquero-Siguero[1], Quanhua Mu[2], Paula Kroon[1], Ying Zhang[3], Marcos Galán-Ganga[1], Zhaoshi Bao[2,3], Zheng Wang[3], Hanjie Liu[3], Junfei Zhao[4], Kim Hoon[5], Sandra Rodriguez-Perales[6], Roel GW Verhaak[5], Raul Rabadan[4], Tao Jiang[3], Jiguang Wang[2] and **Massimo Squatrito[1]**

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Temozolomide (TMZ) is an oral alkylating agent used for the treatment of glioblastoma and is now becoming a chemotherapeutic option in patients diagnosed with high-risk low-grade gliomas1. The O-6-methylguanine-DNA methyltransferase (MGMT) is responsible for the direct repair of the main TMZ-induced toxic DNA adduct, the O6-Methylguanine lesion. MGMT promoter hypermethylation is currently the only known biomarker for TMZ response in glioblastoma patients2. Here we show that a subset of recurrent gliomas carry MGMT genomic rearrangements that lead to MGMT overexpression, independently from changes in its promoter methylation. By leveraging the CRISPR/Cas9 technology we generated some of these MGMT rearrangements in glioma cells and we demonstrated that they lead to TMZ resistance both in vitro and in vivo. Lastly we showed that such fusions can be detected in tumor-derived exosomes and could potentially represent an early detection marker of tumor recurrence in a subset of patients treated with TMZ.

Cancer drivers and dependencies

Scott W. Lowe
Howard Hughes Medical Institute,
Memorial Sloan Kettering Cancer Center,
New York, US

Our laboratory is interested in understanding how the genetic alterations in cancer cells contribute to tumorigenesis, alter treatment response, and create vulnerabilities that may be targeted therapeutically. Historically our focus has been on studying tumor suppressor genes, particularly in the processes of cell death and senescence, and we have established the importance of these processes in tumor suppression and therapy response. We also explore the genetic requirements for tumor maintenance in order to identify new cancer targets. To facilitate our research, we combine genetic and genomic tools that enable us to explore various aspects of cancer biology in a comprehensive way. We also developed fast and flexible non-germline mouse models to accelerate our ability to probe gene function in vivo, and have used these to characterize new cancer drivers and dependencies. In this lecture, I will describe efforts to using novel mouse models to interrogate p53 action and the consequences of p53 mutations during tumorigenesis. I also will discuss new mouse modeling strategies aimed at interrogating the origins and implications of cancer heterogeneity.

Madrid 23 — 25 Sept 2019

Heterogeneity and Evolution in Cancer

24th September 2019 — Tuesday

Session #3

Chairperson: ***Scott Lowe***

The statistical ensemble approach to adaptive immunity

Curtis Callan
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When new cells of the adaptive immune system are created, their DNA undergoes stochastic gene editing to provide the diversity needed to deal with pathogens. Copious data on this diversity are being provided by high-throughput sequencing. We describe a statistical inference framework that uses such data to quantify hidden statistical aspects of the immune system, such as the probability that any specific T cell sequence will be generated in a stem cell event. It turns out that these probabilities range over many orders of magnitudes for typical human T cell repertoires, a phenomenon that underscores the necessity of a statistical approach to the immune system. More generally, a statistical ensemble point of view enables novel modes of analyzing immune function and may have implications for immunotherapy. Emerging cancer data sets provide an ideal field for application of analyses based on these ideas.

Mutant p53 activities in somatic models of osteosarcoma and breast cancer

Guillermina Lozano
The University of Texas MD Anderson Cancer Center,
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Disruption of the p53 tumor suppressor pathway commonly occurs via missense mutations, many of which exhibit gain-of-function activities such as increased tumor aggressiveness and metastasis. We have developed novel conditional mutant *p53* alleles that switch wild type p53 to mutant in a Cre-specific manner to explore the role of the microenvironment in tumor development and progression. Somatic models of osteosarcoma and breast cancer driven by p53 missense mutations will be discussed. These models most closely simulate the genesis of somatic cancers and will thus be invaluable in testing novel therapeutic combinations.

SHORT TALK

Predicting tumor evolution using cancer progression models

Ramón Díaz-Uriarte, Claudia Vasallo

Dept. Biochemistry, Universidad Autónoma de Madrid and
Instituto de Investigaciones Biomédicas "Alberto Sols" (UAM-CSIC), Madrid, Spain

Successful prediction of the likely paths of tumor progression is valuable for diagnostic, prognostic, and treatment purposes. Cancer progression models (CPMs) use cross-sectional samples to identify restrictions in the order of accumulation of driver mutations and thus encode the paths of tumor progression. Here we examine whether CPMs can be used to predict the true distribution of tumor progression paths and to estimate evolutionary unpredictability. Employing simulations we show that if fitness landscapes are single peaked (have a single fitness maximum), there is good agreement between true and predicted distributions of evolutionary paths when sample sizes are large, but performance is poor with the currently common much smaller sample sizes. Under multi-peaked fitness landscapes (i.e., those with multiple fitness maxima), performance is poor and improves only slightly with sample size. In all cases, detection regime (when tumor samples are taken) is a key determinant of performance. Estimates of evolutionary unpredictability from CPMs tend to overestimate the true unpredictability and the bias is affected by detection regime; CPMs could be useful for estimating upper bounds to the true evolutionary unpredictability. Analysis of twenty two cancer data sets shows estimates of evolutionary unpredictability in regions where useful prediction might be possible for at least some data sets. But most of the evolutionary trajectory predictions themselves are very unreliable, and unreliability increases with the number of features analyzed. Our results indicate that CPMs could be valuable tools for predicting cancer progression but that, currently, obtaining useful predictions of tumor progression paths from CPMs is dubious, and emphasize the need for methodological work that can account for the probably multi-peaked fitness landscapes in cancer.

Quantifying tumor evolution across time and space

Benjamin J. Raphael
Department of Computer Science,
Lewis-Sigler Institute for Integrative Genomics,
Princeton, US

Somatic mutations provide markers to infer the ancestral relationships between cells of a tumor and to describe the patterns of cellular migrations between a primary tumor and metastases at distant anatomical sites. However, such phylogenetic analyses are complicated by specific features of cancer sequencing data including: heterogeneous mixtures of cells in bulk tumor sequencing data, undersampling and errors in single-cell sequencing data, and large-scale genome rearrangements and copy number aberrations. I will describe computational approaches to study clonal heterogeneity and evolution using bulk tumor and single-cell DNA sequencing data. I will present algorithms to: infer seeding patterns of metastases and distinguish monoclonal vs. polyclonal seeding; leverage temporal information in longitudinal samples to overcome ambiguity in bulk tumor sequencing data; integrate single-nucleotide mutations and copy number aberrations in single-cell sequencing data. I will demonstrate the application of these algorithms to infer evolutionary histories in multiple cancer types.

Heterogeneous Immune Determinants of Tumor Evolution

Benjamin D. Greenbaum
Icahn School of Medicine at Mount Sinai,
New York, US

Immune recognition is a critical determinant of pathogen evolution, and avoidance of immune detection is a critical issue for a tumor. It has become clear that interventions which can properly engage the immune system can fundamentally alter tumor evolution. However, which alterations alter the evolutionary trajectory of a tumor or lead to actionable therapeutic outcomes remains unclear. A key mathematical issue that has emerged is therefore the extent to which we can quantify the features that make a molecule immunogenic and which of those features can be a fitness determinant either endogenously or under therapy. Here we will discuss which immunological features are associated with different aspects of tumor evolution and may lead to actionable outcomes. We discuss a set of methods used to quantify evolutionary pressures exerted by immune recognition and how to insert these immunological features into evolutionary models. We will discuss our recent work on this problem where we have learned from the genome evolution of tumors and viruses, and its implications for cancer immunotherapies.

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Session #4

Chairperson: ***Fátima Al-Shahrour***

Age related mutational burden in human colonic epithelium is dictated by stem cell renewal processes

Douglas J. Winton
Cancer Research UK Cambridge Institute,
Cambridge, UK

To cause cancer oncogenic mutations must become permanently fixed within tissues. Understanding the origins of mutational burden has, to date largely focused on somatic mutation rates and the role of intrinsic cellular properties versus exogenous sources of mutagens. However, in the colonic epithelium mouse studies indicate that stem cell replacement and expansion processes make a major determinant of both the probability of mutations becoming fixed and their subsequent representation within the tissue.

Building on approaches developed in mouse we investigated stem cell renewal processes in human colonic epithelium. Visualizing somatic clones that arise by spontaneous somatic mutation reveals a linear mode of age related accumulation for neutral mutations. Modelling the effects of gene mutation, stem cell dynamics and subsequent lateral expansion indicates that fixation requires two sequential steps. Initially, one of a small number (between 5 and 10) of active stem cells residing within each colonic gland has to be mutated. Subsequently, the mutated stem cell has to 'win' in stochastic competition with its neighbours to populate the entire gland. This process takes many years (median 6.3) for neutral mutations because stem cell replacement is infrequent. Subsequent clonal expansion arising from the fission or replication of glands is also rare for neutral mutations with the size of mutant fields consequently remaining small. In contrast to neutral events, biased or pro-oncogenic mutations can subvert both stem cell replacement to accelerate fixation and clonal expansion by gland fission to achieve high mutational burden with age. The extreme case of Kras activating mutations is considered. Overall the benchmarking and quantification of these behaviours demonstrates a major contribution of stem cell renewal processes in dictating age-related mutant allele frequencies. Further they allow the advantage associated with different gene specific mutations to be compared and ranked irrespective of the cellular mechanisms by which they are conferred. The age-related mutational burden of advantaged mutations can be predicted on a gene-by-gene basis to identify windows of opportunity to affect fixation and limit spread.

Tumor genomes shed light into somatic mutational processes and cancer vulnerabilities

Nuria Lopez-Bigas
Institute for Research in Biomedicine,
Barcelona, 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 DNA-binding proteins, nucleosomes and differences in exons and introns influence 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 genomic elements involved in cancer is by tracing the signals left by the positive selection of driver mutations across tumours. We analyze thousands of tumor genomes to identify driver mutations in coding and non-coding regions of the genome. The analysis of tumor cohorts provide valuable information to improve the interpretation of individual variants detected in newly sequenced tumors in clinical or research settings. We have developed CancerGenomeInterpreter.org, a tool designed to identify driver mutations and biomarkers of drug response in individual tumors.

SHORT TALK

Intratumour heterogeneity in a pancreatic cancer mouse model

Laura Gonzalez-Silva[1], Laura Quevedo[1], Thaidy Moreno[1], Carlos Revilla[1], Dieter Saur[2,3,4,5], Roland Rad[2,4,5] and Ignacio Varela[1]

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Intratumour heterogeneity has been observed in multiple cancers and has been postulated as a critical aspect for tumour metastasis and treatment resistance. Therefore, a further characterization of its role in cancer progression and metastasis has become essential. Pancreatic cancer in humans has a dismal prognosis with only 8% of patients surviving more than 5 years after diagnosis. Mouse models of pancreatic cancer, based on the expression of an oncogenic version of Kras in the pancreas, have been widely used to study the molecular pathways involved in pancreatic cancer progression, nevertheless there is still controversy about their utility to study the genetic complexity observed in human tumours.

We have performed multi-sampling exome, genome and single-cell wholetranscriptome sequencing in primary and metastatic lesions, as well as in primary cell cultures generated from an oncogenic Kras-mediated mouse model of pancreatic cancer.

We have observed that murine tumours recapitulate the genetic complexity observed in the human tumours like a monofocal origin of the aggressive disease, multi-clonal intratumour heterogeneity, independent waves of metastatic colonization with clone convergent evolution and the presence of chromotripsis. In addition, we have been able to characterize the tumour microenvironment and the presence of diverse cell populations, such as CAFs and immune cells. Our results show that the oncogenic Kras-based mouse model is a very good tool for the study of the dynamics of intratumour heterogeneity and that it also reflects faithfully the genomic processes observed in human pancreatic tumours. Consequently, we propose that a deeper genomic and functional study of these murine tumours could provide a better understanding of the role of intratumour heterogeneity in tumour progression and metastasis.

Clonal evolution mechanisms in leukemia initiation and relapse

Adolfo Ferrando

Professor of Pediatrics, Pathology & Cell Biology and Systems Biology,
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New York, US

Acute lymphoblastic leukemia is an aggressive hematologic malignancy resulting from the cancerous transformation of hematopoietic precursors with block in differentiation in the early stages of lymphoid development. Genomic studies have dissected the genetic landscape of this disease pointing to driver oncogenic lesions. Moreover analysis of diagnostic and relapsed leukemias shows marked shifts in mutational landscape, branched clonal evolution and gain of chemotherapy resistance associated genetic alterations at relapse. Here we will discuss the role of clonal dominance at the hematopoietic stem cell level in leukemia initiation and the organization of intratumor stem cell architecture and the role of chemotherapy-driving mutations in disease progression and relapse.

Measurement of selective coefficients for subclonal mutations in human cancer

Trevor Graham
Barts Cancer Institute,
London, UK

Quantitative measurement of the evolutionary dynamics of cancer subclones enables future dynamics to be extrapolated, with implications for early detection and treatment optimisation. I will discuss how fitness advantage of subclones can be inferred from routinely available genome sequencing data through fitting of mathematical models that describe subclonal evolution to those data. First, I will describe an approach to infer fitness advantages on a tumour-by-tumour basis through direct inference on the site frequency spectrum (variant allele frequency distribution) [1]. Second, I will discuss the signature of immune-mediated negative selection in the site frequency spectrum [2]. Third, I will describe how dN/dS measurements (the normalised ratio of non-synonymous to synonymous mutations; a commonly used measure of selection in evolutionary biology) can be adapted to cancer evolution to reveal average subclone fitness advantage in large tumour cohorts [3]. Together, these approaches reveal that tumour subclones can experience remarkably large increases in fitness (>20%) and highlight the problems inherent to quantifying negative selection in cancer genomes.

[1] https://doi.org/10.1038/s41588-018-0128-6
[2] https://doi.org/10.1101/536433
[3] https://doi.org/10.1101/661264

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Heterogeneity and Evolution in Cancer

24th September 2019 — Tuesday

Session #5

Chairperson: ***Solip Park***

Complex roles of p53, Mdm2 and MdmX in survival and death of cancer cells

Carol Prives

Department of Biological Sciences, Columbia University,
New York, US

Despite years of intense scrutiny there are still many unknown features concerning p53 or its negative regulators Mdm2 and MdmX in terms of their varied roles in cells. Regarding p53, our group has discovered novel transcriptional targets of p53 that mediate its roles in some forms of cancer. For example, in collaboration with the group of Scott Lowe we discovered that ABCA1 is a p53 target gene that mediates the ability of p53 to dampen the expression of the mevalonate pathway and that this plays a role in suppression of hepatocellular carcinoma in a mouse model. Further, with Raul Rabadan we have discovered a set of long non-coding (lnc) RNAs that are likely p53 target genes and that levels of some of these lnc RNA targets are correlated with patient survival data in some forms of cancer. Interestingly there is little or no overlap between different p53-regualted lnc RNAs in cancers originating from different tissue types.

With respect to Mdm2 and MdmX we have identified both survival and death roles of these two proteins that are independent of p53. Depletion or inhibition of either or both of these proteins in cells lacking p53 or harboring solely mutant p53 leads to cell cycle arrest and in some cases cell death. Yet, in collaboration with Brent Stockwell and colleagues, after initiation of the process of ferroptotic cell death, we have discovered that both Mdm2 and MdmX are actually required for this form of cell death. In cells undergoing ferroptosis Mdm2 and MdmX are play roles in rewiring of cellular lipids and counteracting the anti-oxidant defenses of the cells.

Our findings thus highlight the need to examine p53, Mdm2 and MdmX in both different cellular contexts and cancer cell environments.

Authors: Carol Prives, Bita Alaghebandan, Ana Maria Low Calle, Joshua Choe, Nicholas Anthony O'Brien, Mercedes Fissore-O'Leary, Chen Katz, Alyssa Klein, Sung-Hwan Moon, Rafaela Muniz, Kausik Regunath, David Tong and Divya Venkatesh

Organizing spatial and temporal intratumoral heterogeneity on microscopic tissue sections

Marc Ryser[1] and **Darryl Shibata[2]**

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Multiple subclones are common in human tumors. This intratumoral heterogeneity (ITH) could represent clonal evolution where subclones with greater fitness confer more malignant phenotypes. Alternatively, ITH could represent the expansion of subclones of similar fitness marked by different passenger mutations. Here we show that phenotypic, ancestral, and topographic information can be merged after saturation microdissection and deep resequencing of tumor sections. By localizing subclones directly onto microscopic sections it is possible to infer the ancestry of each tumor region and the final phenotypes of the subclones. We show that multiple mm sized subclones are commonly present within single tumor sections and they are jigsaw arrayed in vertical columns where subclones share invasive and superficial phenotypes. The phylogeography of ITH of many human colorectal tumors are consistent with single, effectively neutral "Big Bang" expansions where the founder cell starts with the driver mutations and the phenotypic plasticity to rapidly expand into a visible tumor.

SHORT TALK

Inference of mutational heterogeneity and variant clonality reveals distinct genomic landscape for hematopoietic cells admixed in solid tumor microenvironment

Gregory Riedlinger, Mohammad Hadigol, Shridar Ganesan and **Hossein Khiabanian**

Rutgers Cancer Institute of New Jersey, Rutgers University,
New Brunswick, NJ, US

Recent advances in clinical sequencing have resulted in unprecedented access to the genomes of individual tumors. These assays aim to identify somatic mutations in cancer cells; however, genomic heterogeneity in both tumor and non-tumor cell populations confounds distinguishing subclonal tumor alterations from those possibly originating from the non-tumor component of the tumor microenvironment. We have developed information-theoretic algorithms that permit selecting the most consistent model for variants' mutational status to infer loss of heterozygosity, mutated allele's copy-number, and cancer cell fraction, while accounting for biases inherent to clinical settings. Our analysis of high-depth sequencing of 2,030 specimens from patients with solid tumors showed that some detected mutations arose from hematopoietic cells infiltrating the tumor microenvironment. In addition to the presence of mutations associated with coexistent hematological malignancies, some mutations were detected due to an age-related condition known as clonal hematopoiesis of indeterminate potential (CHIP). By sequencing peripheral blood and macrodissected lymphocytes from solid tumor patients, we showed that 79% of subclonal CHIP-associated mutations found in the original specimens were not tumor mutations, but instead were detected due to the presence of CHIP. Our extended analysis of 113,079 solid tumors from 21 cancer types further demonstrated the prevalence of CHIP and illustrated its differential genomic landscape when enriched in solid tumor microenvironment. These results raise the hypothesis that therapeutics directed at the solid tumor may produce cross-sectional changes in CHIP's evolutionary patterns. To characterize extrinsic selection pressures that may drive CHIP's transformation to leukemia, there is a need for molecularly defined longitudinal analysis of hematopoietic populations that identify patients at risk of developing therapy-related hematological complications.

Rational therapeutic combination with BH3 mimetics to overcome cancer adaptation to treatment

SHORT TALK

Clara Alcon[2], Albert Manzano[2], Cécile Gstadler[1], Dorota E. Sadowicz[1], Anthony Letai[1], Josep Samitier[2], Rizwan Haq[1], **Joan Montero[1,2]**

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In the clinic, it is often observed that cancer patients first respond to targeted agents showing tumor burden shrinkage but subsequently relapse with tumors that are more aggressive and no longer responsive to therapy, frequently acquiring an antiapoptotic defense. However, the problem is to correctly to determine and assign the best therapy for every patient to overcome this resistance. We previously developed a functional predictive assay called dynamic BH3 profiling (DBP) that can rapidly assess cytotoxic response to therapy. This strategy has been extensively utilized in many cancer models, also on patient samples, with outstanding results. From an innovative point of view, we use DBP with selective BH3 peptides to determine the tumors' defense adaptation over time and guide the use of BH3 mimetics (small molecules that inhibit BCL-2 family antiapoptotic proteins) to overcome resistance and restore cell death.

Our first set of experiments consisted in determining DBP's predictive capacity in melanoma cell lines, showing that it is a good binary predictor, based on ROC curve analyses. We next characterized the tumor's adaptation to therapy over time. We performed DBP over time, and we observed that when they were effectively treated with BRAF or MEK inhibitors there was an increased dependence in MCL-1 starting at 36 hours. This observation explains a possible mechanism by which melanomas become resistant to first-line treatment, adapting their antiapoptotic strategy to overcome chemotherapy. Based on these observations, we tested a sequential therapeutic combination consisting on pretreating the cancer cells with dabrafenib and then adding a specific MCL-1 inhibitor. This strategy worked both *in vitro*, causing a cytotoxicity >90%, and *in vivo* in a xenograft model showing that this metronomic combination eliminated the tumors. Similar results were found in two of the most common pediatric cancer, rhabdomyosarcoma and B-cell acute lymphoblastic leukemia.

Our results with cell lines and in vivo models demonstrate DBP's potential to be used as a powerful real-time tool to study combinations of anticancer agents with BH3 mimetics to better treat therapy-resistant tumors, both adult and pediatric, in the clinic.

Delineating the rates and routes of metastasis

Christina Curtis
Stanford University, School of Medicine,
Stanford, US

Metastasis is the most lethal and insidious aspect of cancer. Despite significant therapeutic advances, metastatic disease is generally incurable. To date, the molecular and microenvironmental determinants of metastasis are largely unknown, as is the timing of systemic spread, hindering effective treatment and prevention efforts. In this talk, I will describe several quantitative frameworks to delineate the dynamics of distant metastasis and their application to different solid tumors and types of cohorts. First, I will outline a suite of computational tools we have developed to infer the evolutionary dynamics of tumor progression from patient genomic data by coupling population genetic theory, spatial computational modeling and approximate Bayesian computation. Building on these efforts, I will describe a new method, termed SCIMET (Spatial Computational Inference of METastatic Timing) to infer the timing of metastatic spread based on patterns of genomic divergence between paired primary tumors and distant metastases (Hu et al. *Nature Genetics* 2019). I will show how application of this approach to colorectal cancer enables quantification of the rates and routes of metastasis in a patient-specific fashion and yields fundamental insights into the drivers of this lethal process with attendant clinical implications and extensions of this framework to other tumor types. Lastly, I will describe a statistical approach to model the dynamics of breast cancer relapse and application to a cohort of nearly 2000 breast cancers with detailed genomic information and long-term clinical follow-up (Rueda et al. *Nature* 2019). Throughout, I will discuss context dependencies that underlie disease progression and how this may inform strategies for patient stratification and therapeutic targeting.

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Heterogeneity and Evolution in Cancer

25th September 2019 — Wednesday

Session #6

Chairperson: ***Núria López-Bigas***

Exploiting evolutionary steering to control drug resistance in cancer

Andrea Sottoriva
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Drug resistance mediated by clonal evolution is arguably the biggest problem in cancer therapy today. However, evolving resistance to one drug may come at a cost of decreased growth rate or increased sensitivity to another drug due to evolutionary trade-offs. This weakness can be exploited in the clinic using an approach called 'evolutionary steering' that aims at controlling the tumour cell population to induce collateral drug sensitivity and delay resistance. However, recapitulating cancer evolutionary dynamics experimentally remains challenging. Here we present a novel approach for evolutionary steering based on a combination of single-cell barcoding, very large populations of 10^8–10^9 cells grown without re-plating, longitudinal non-destructive monitoring of cancer clones, and mathematical modelling of tumour evolution. We demonstrate evolutionary herding in non-small cell lung cancer, showing that steering allows shifting the clonal composition of a tumour in our favour, leading to collateral drug sensitivity and decreased fitness due to proliferative costs. Through genomic analysis and single-cell RNA sequencing, we were also able to determine the mechanisms that drive such evolved sensitivity. Our approach allows modelling evolutionary trade-offs experimentally to test patient-specific evolutionary steering strategies that can potentially be translated into the clinic to control treatment resistance.

Immune interactions predict cancer evolution

Marta Łuksza
Icahn School of Medicine, Mount Sinai,
New York, US

In recent years, novel therapies for treating cancer by means of a patient's own immune system have emerged. Checkpoint-blockade immunotherapies are designed to enable immune system cells to recognize and destroy cancer cells. The process of recognition is based on specific protein binding interactions between the immune and cancer cells. Because these interactions depend on mutations in the cancer genome, immune recognition is also an evolutionary problem. I will present a new mathematical model of cancer evolution based on the fitness cost of tumor cells due to immune recognition. The model successfully predicts response to checkpoint-blockade immunotherapy, as shown in patient cohorts with melanoma and lung cancer. Our results highlight evolutionary similarities between cancer and viral pathogens and suggest general concepts of predictive analysis in fast-evolving systems.

SHORT TALK

Characterizing transcriptional and genomic heterogeneity in circulating tumor cells

Francesc Castro-Giner[1,2], Katharina Jahn[2,3], Barbara Szczerba[2], Jack Kuipers[2,3], Francesco Marass[2,3], Jochen Singer[2,3], Niko Beerenwinkel[2,3], Nicola Aceto[2]

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The analysis of circulating tumor cells (CTCs) at single-cell resolution offers a great promise for the understanding of the biology and vulnerabilities of the metastatic process. Similarly to primary tumors, CTCs are likely to be characterized by interpatient and intrapatient heterogeneity, both at the level of gene expression and at the mutational level. Recent developments in the field of single-cell sequencing technologies provide the possibility of parallel analysis of multiple omics dimensions from the same cell. In order to examine the relationship between genetic and transcriptional heterogeneity of CTCs, we performed a comprehensive characterization of CTCs from both breast cancer patients and xenografts, using single-cell exome and transcriptome analysis. We identified transcriptional subpopulations of CTCs, some of them shared by multiple individuals, and examined their relationship with genetic subclones identified through phylogenetic analysis. Our results provide insights into genetic and transcriptional heterogeneity of CTCs, highlighting potentially important aspects of the metastatic process.

SHORT TALK

Phenotypic heterogeneity (not genetic) underpins survival during induction chemotherapy in childhood acute lymphoblastic leukaemia

Virginia A. Turati[1], José Afonso Guerra-Assunção[1], Chuling Ding[1], Nicola E. Potter[2], Simone Ecker[1], Nicola E. Potter, Rajeev Gupta[1], Amy Webster[1], Maxime Tarabichi[3], Agne Danevichute[1], Georgina W. Hall[4], Andrea Biondi[5], Giovanni Cazzaniga[6], Lisa Russell[7], Mark Lynch8, Peter Van Loo[3,9], Iain Macaulay[10], Mike Hubank[11], Sten E. Jacobsen[12,13], Mel Greaves[2], Javier Herrero[1], Tariq Enver[1*]

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[10]The Earlham Institute, Norwich, UK
[11]The Institute of Child Health, London, UK
[12]Weatherall Institute of Molecular Medicine, University of Oxford, UK
[13]Karolinska Institutet, Stockholm, Sweden

Those rare cancer cells that persist immediately after chemotherapy provide the substrate for subsequent disease evolution and later clinical events. We investigated selection mechanisms operating immediately post-chemotherapy using a mouse xenograft model, whereby individual childhood B-cell acute lymphoblastic leukaemias were transplanted into multiple recipients, which then received identical conventional chemotherapy, allowing us to distinguish stochastic versus deterministic events. We compared genomic and transcriptional heterogeneity in residual leukemic cells at the single cell level and observed that, contrary to prevailing notions, chemotherapy has little selective impact on intra-tumour genetic heterogeneity. Rather the focus of selection is the extensive inter-cellular transcriptional variability present within the untreated disease. After chemotherapy, a phenotypically uniform population remains - characterized by primitive developmental stage (increased expression of multi- lymphoid/proB-cell features) and a newly defined deep quiescence phenotype (diminished expression of c-MYC, E2F and their target genes). These cells also functionally display increased tumour initiating potential. The findings of our genomic and transcriptome analysis reconcile in the observation that quiescent early B-cell progenitors at diagnosis are polyclonal. Finally, genome-wide methylation arrays showed that in agreement with the transcriptome data, resistant cells are, at the population level, characterized by more uniform methylation patterns compared to untreated cells; therefore implying that aberrant DNA methylation status is a key contributing factor to chemoresistance. We conclude that epigenetic state is the principal target for selection of resistant disease during chemotherapy.

Epigenetic rewiring and tumor plasticity

Christina Leslie
Sloan Kettering Institute,
Memorial Sloan Kettering Cancer Center,
New York, US

Dysregulated epigenetic programs are a feature of many cancers, and the diverse differentiation states of immune cells as well as their dysfunctional states in tumors are in part epigenetically encoded. We will present new analysis work and computational methodologies from our lab to decode epigenetic programs from genome-wide data sets.

In a recent collaborative work, we characterized chromatin states governing CD8 T cell dysfunction in cancer and reported that tumor-specific T cells differentiate to dysfunction through two discrete chromatin states: an initial plastic state that can be functionally rescued (i.e. through immunotherapy) and a later fixed state that is resistant to therapeutic reprogramming. We now follow up on this work by presenting a computational methodology to decipher transcriptional programs governing chromatin accessibility and gene expression in normal and dysfunctional T cell responses through a large-scale analysis of published data from mouse tumor and chronic viral infection models. This modeling shows that in all these systems, T cells commit to becoming dysfunctional early after an immune challenge, rather than first mounting and then losing an effector response. Through scRNA-seq analysis, we characterize the phenotypic diversity of this common trajectory from plastic to fixed dysfunction.

We will also present a recent collaboration with the Sawyers lab on FOXA1 mutants in prostate cancer, showing that somatic alterations in this pioneer transcription factor lead to altered differentiation programs, through analysis of ATAC-seq and ChIP-seq in mouse prostate organoid systems.

Mathematical modeling of cancer heterogeneity

Ivana Bozic
University of Washington,
Washington, US

Cancer is the result of a stochastic evolutionary process characterized by the accumulation of mutations that are responsible for tumor growth, immune escape, and drug resistance, as well as mutations with no effect on the phenotype. Stochastic modeling can be used to describe the dynamics of tumor cell populations and to obtain insights into the hidden evolutionary processes leading to cancer. I will present recent approaches that use branching process models of cancer evolution to quantify intra-tumor heterogeneity and the development of drug resistance, and their implications for interpretation of cancer sequencing data and the design of optimal treatment strategies.

Evolutionary therapy for metastatic cancer

Alexander R. A. Anderson

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Our current approach to precision medicine is dictated by finding molecular targets, those patients fortunate enough to have a targetable mutation will receive a fixed treatment schedule designed to deliver the maximum tolerated dose. These therapies generally achieve impressive short-term responses, that unfortunately give way to treatment resistance and tumor relapse. Using an integrated mathematical/experimental/clinical approach we illustrate the importance of using treatment response as a key driver of future treatment decisions, rather than fixed strategies that ignore it. Our evolutionary therapeutic approach explicitly accounts for underlying tumor and microenvironmental heterogeneity and exploits it through the principle of competitive release. This assumes that for drug resistant cells, benefits often exceed costs during therapy, however, in the absence of therapy, drug sensitive cells are fitter due to the cost of resistance. Treatment holidays can exploit this cost by allowing sensitive cells to outcompete their resistant counterparts. Even in the absence of such a cost, combination therapies given more strategically (e.g. sequentially, with or without holidays) can delay the emergence of resistance. Our results strongly indicate that the future of precision medicine shouldn't be in the development of new drugs but rather in the smarter evolutionary application of preexisting ones.

Inferring microbial activity from metagenomic data

Tal Korem
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New York, US

The gut microbiome is an immense microbial ecosystem with unique and diverse metabolic capabilities. In the past decade, it has been associated with multiple chronic and complex diseases, including cancer, raising great hopes for novel medical advances. But are contemporary microbiome analysis methods useful in a clinical setting? I will present new tools that we developed for microbiome analysis of the gut microbiome that utilize genomic sequencing coverage to yield biological and mechanistic insights about the microbiome in the context of health and disease. I will additionally present potential applications in a new research project aimed to identify novel risk factors for pancreatic cancer.

Madrid 23 — 25 Sept 2019

Heterogeneity and Evolution in Cancer

Organisers & Speakers' Biographies

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Fátima Al-Shahrour
Head of Bioinformatics Unit
Spanish National Cancer Research Centre,
Madrid, Spain

Fátima Al-Shahrour PhD, is head of the Bioinformatics Unit (BU, https://bioinformatics.cnio.es/) at Spanish National Cancer Research Centre. Her research focuses on applying and developing computational methods to precision medicine, for the interpretation of cancer genomes, drug repositioning and prediction of anticancer therapies. Her main goal is to provide the framework and expertise in order to identify new biomarkers and mechanisms of drug response in cancer. Her main goal is to translate this knowledge into effective treatments for cancer patients.

Her Unit belongs to National Bioinformatics platform (*Instituto Nacional de Bioinformática - ISCIIII*) and ELIXIR-ES as an emergent node (https://inb-elixir.es/). She is co-director of Master in Bioinformatics Applied to Personalized Medicine and Health *Instituto de Salud Carlos III* (INS-ISCIII).

Alexander R. A. Anderson
Moffitt Cancer Center and Research Institute,
Tampa, US

Alexander R. A. Anderson, PhD is chair of the Integrated Mathematical Oncology (IMO) Department at Moffitt Cancer Center. Most of his early cancer work at Dundee university was on developing mathematical models of tumor progression and treatment, including anti-angiogenesis, radiotherapy, tumor invasion, intra-tumor heterogeneity evolution. Due to his belief in mathematical models in cancer research he moved to Moffitt in 2008 to establish the IMO. At Moffitt his research shifted to developing organ specific models of tumor initiation and progression, examine the key role of the microenvironment as a selective force in cancer evolution. Recently, clinical translation has become central to his research leading to the development of eco-evolutionary therapies that seek to control cancer rather than eradicate it.

Niko Beerenwinkel
ETH Zürich, Zürich,
Switzerland

Niko Beerenwinkel is full professor of computational biology at the Department of Biosystems Science and Engineering of ETH Zurich in Basel. His research is at the interface of mathematics, statistics, and computer science with biology and medicine. It includes the development of statistical models for high-throughput molecular profiling data, network-based analysis of genome-wide perturbation screens, evolutionary modeling, and clinical applications in oncology and infectious diseases. He has developed a computational pipeline for the molecular tumor board Zurich, including methods for the analysis of single-cell sequencing data, and algorithms and tools for mining viral genomes and improving clinical diagnostics of viruses.

Ivana Bozic
Assistant Professor
Department of Applied Mathematics
University of Washington, Seattle
Washington, US

Dr. Bozic is an Assistant Professor of Applied Mathematics at the University of Washington in Seattle. She obtained her Ph.D. from Harvard University in 2012, where she continued to do her postdoc in mathematical biology. Dr. Bozic studies the evolution if cancer and its resistance to treatment using mathematical models and clinical data.

Curtis G. Callan
Physics Department, Princeton University,
Princeton, US

When new cells of the adaptive immune system are created, their DNA undergoes stochastic gene editing to provide the diversity needed to deal with pathogens. Copious data on this diversity are being provided by high-throughput sequencing. We describe a statistical inference framework that uses such data to quantify hidden statistical aspects of the immune system, such as the probability that any specific T cell sequence will be generated in a stem cell event. It turns out that these probabilities range over many orders of magnitudes for typical human T cell repertoires, a phenomenon that underscores the necessity of a statistical approach to the immune system. More generally, a statistical ensemble point of view enables novel modes of analyzing immune function and may have implications for immunotherapy. Emerging cancer data sets provide an ideal field for application of analyses based on these ideas.

Neal G. Copeland

Neal G. Copeland, Ph.D.
Professor of Practice
University of Texas MD Anderson Cancer Center,
Houston, Texas, US

Neal Copeland received his PhD from the University of Utah and was a postdoctoral fellow at Harvard Medical School, where he met his wife and long-time collaborator, Nancy Jenkins. During their more than 40 years together, they worked at the Jackson Laboratory (Maine), National Cancer Institute (Maryland), Institute of Molecular and Cell Biology (Singapore) and MD Anderson Cancer Center (Texas). They have modeled many different types of human disease in the mouse, but have focused exclusively on cancer during the last 13 years. They have published more than 800 papers together and are both members of the National Academy of Sciences (USA).

Christina Curtis
Stanford University School of Medicine,
Stanford, US

Dr. Christina Curtis is faculty at Stanford University School of Medicine, where she leads the Cancer Computational and Systems Biology Group and is Co-director of the Molecular Tumor Board. Dr. Curtis' research is focused on delineating mechanisms of tumor progression and developing biomarkers to advance precision medicine. Her research has led to new paradigms in understanding how human tumors evolve and has redefined the molecular taxonomy of breast cancer, yielding potential new therapeutic targets. Dr. Curtis is a National Academy of Sciences Kavli Frontier of Science Fellow and recipient of the 2018 NIH Director's Pioneer Award.

Adolfo A. Ferrando
Institute for Cancer Genetics, Columbia University,
New York, US

My group at the Institute for Cancer Genetics at Columbia University works on the genetic and molecular basis of acute lymphoblastic leukemia and non-Hodgkin lymphoma using a combination of genomic technologies, biochemical and genetic analysis. Over the last 14 years we have successfully used an integrated approach combining genomics, transcriptomics, biochemical assays and animal models to analyze the functions of key oncogenes including NOTCH1 (PNAS 2006; Nat Med 2007, PNAS 2009; Nat Med 2009, Nat Med 2014, Nat Med 2015, PNAS 2017) and the TLX1 and TLX3 oncogenes (Nat Med 2010, Nat Med 2012), in the pathogenesis of T-ALL. In addition, we have identified and functionally characterized numerous genes somatically mutated in this disease including *PTEN* (Nat Med 2007, Cancer Cell 2013), *WT1* (Blood 2009), *PHF6* (Nat Genet 2010, Cancer Disc 2018), *BCL11B* (Nat Med 2010), *ETV6* (J Exp Med 2011), *EZH2* (Nat Med 2012b) and *NT5C2* (Nat Med 2013, PNAS 2016, Nature 2018, Cancer Cell 2018) as well as in peripheral T-cell lymphomas (Nat Genet 2014, Nat Genet 2015, PNAS 2017). Over the last years we have built an extensive network of collaborators. As PI or co-Investigator on several previous grants funded by the NIH, the Leukemia and Lymphoma Society and other private foundations, I have built a highly trained and well-coordinated team with specific expertise in genomics, bioinformatics, protein biochemistry, genetically manipulated mouse models of leukemia and experimental therapeutics. These projects have produced numerous peer-reviewed publications from my group and in collaboration with other researchers.

Trevor Graham
Barts Cancer Institute,
London, UK

Professor Trevor Graham leads the Evolution and Cancer Laboratory at the Barts Cancer Institute, QMUL, London UK. The lab combines mathematical theory, computational biology and wet-lab measurements (mainly genomics) to study the dynamics of tumour evolution. Trevor's background is in mathematics (PhD Mathematical Biology UCL, 2009), with postdoctoral training in cancer labs (Prof Sir Nick Wright, CRUK London Research Institute 2008-2011 and Prof. Carlo Maley, University of California at San Francisco 2011-2013).

Benjamin D. Greenbaum

Benjamin D Greenbaum, PhD
Assistant Professor,
Icahn School of Medicine at Mount Sinai,
Tisch Cancer Institute,
New York, US

Benjamin Greenbaum, PhD, is an Assistant Professor at the Tisch Cancer Institute at Mount Sinai. He has PhD in theoretical physics from Columbia University and trained at Los Alamos National Laboratory and the Institute for Advanced Study. Dr. Greenbaum utilizes statistical physics, information theory, and evolutionary biology to quantify the interaction of tumors with the immune system, and predict evolutionary trajectories of tumors and viruses. He was awarded a Phillip A. Sharp Award for Innovation in Collaboration from Stand Up to Cancer, a Pershing Square Sohn Prize, a Mark Foundation Fellowship, and named Director of the Center for Computational Immunology.

Holger Heyn

Centro Nacional de Análisis Genómico (CNAG-CRG) - Centre for Genomic Regulation (CRG),
Barcelona Institute of Science and Technology (BIST),
Barcelona, Spain

As leader of the Single Cell Genomics Group at the Spanish National Centre for Genomic Analysis (CNAG-CRG) Dr. Heyn focuses on the systematic integration of single-cell transcriptomics and epigenomics data to elucidate causalities underlying phenotype formation. His group combines technology development with research activities that center on cancer genomics. Heyn's team single-cell approaches are thoroughly benchmarked and standardized to ensure the production of high quality data. Their computational tools allow the analysis of millions of cells and annotation across multiple experiments. Dr. Heyn published 62 articles, including 53 original research articles, 6 Reviews and 3 book chapters. His papers have been 6,724x cited, resulting in an h-index of 37 (Google Scholar). The ERC and Chan Zuckerberg Initiative supports Dr. Heyn's participation in the Human Cell Atlas Project, where he is also co-chairing the Standards and Technology Working Group. Dr. Heyn contributed to several national and international research consortia, including the European Epigenome (BLUEPRINT) and the Cancer Genome Atlas (TCGA) Projects.

Nancy Jenkins
University of Texas MD Anderson Cancer Center,
Houston, Texas, US

Nancy Jenkins received her PhD from Indiana University and was a postdoctoral fellow at Harvard Medical School, where she met her husband and long-time collaborator, Neal Copeland. During their more than 40 years together, they worked at the Jackson Laboratory (Maine), National Cancer Institute (Maryland), Institute of Molecular and Cell Biology (Singapore) and MD Anderson Cancer Center (Texas). They have modeled many different types of human disease in the mouse, but have focused exclusively on cancer during the last 13 years. They have published more than 800 papers together and are both members of the National Academy of Sciences (USA).

Tal Korem

Tal Korem, Ph.D.
Program for Mathematical Genomics, Department of Systems Biology,
Columbia University Irving Medical Center,
New York, US

Tal Korem is an assistant professor at the Program for Mathematical Genomics and the Department of Systems Biology at Columbia University. The ultimate goal of his research is to devise microbiome-based therapeutics and diagnostics. His group is developing methods for microbiome data analysis and approaches for modelling it along with host and environmental data in applied research. He has coauthored several methods for microbiome analysis that infer growth rates of bacteria and their structural variants, as well as an approach for predicting the glycemic responses of individuals to complex meals, using the microbiome.

Christina Leslie

Dr. Christina Leslie
Sloan Kettering Institute
Memorial Sloan Kettering Cancer Center
New York, US

Christina Leslie did her undergraduate degree in Pure and Applied Mathematics at the University of Waterloo in Canada. She was awarded an NSERC 1967 Science and Engineering Fellowship for graduate study and did a PhD in Mathematics at the University of California, Berkeley, where her thesis work dealt with differential geometry and representation theory. She won an NSERC Postdoctoral Fellowship and did her postdoctoral training in the Mathematics Department at Columbia University in 1999-2000. She then joined the faculty of the Computer Science Department and later the Center for Computational Learning Systems at Columbia University, where she began to work in computational biology and machine learning and became the principal investigator leading the Computational Biology Group. In 2007, she moved her lab to Memorial Sloan Kettering Cancer Center, where she is currently a Member of the Computational and Systems Biology Program. Dr. Leslie is well known for developing machine learning approaches – algorithms for learning predictive models from data – for the analysis and interpretation of high-throughput biological data, especially from next-generation sequencing. Her research group uses machine learning and other computational methods to study transcriptional and post-transcriptional gene regulatory mechanisms, epigenetic programs governing cell fate decisions in differentiation, and the dysregulation of gene expression programs in cancer. A strong focus area is the analysis of differentiation programs in the immune system and dysfunctional immune cell states in cancer.

Arnold Levine
The Simons Center for Systems Biology,
Institute for Advanced Study,
Princeton, NJ, US

A. Levine has carried out research in virology and cancer biology. He was among the first to identify the p53 gene and protein and MDM-2, its negative regulator. He helped to elucidate the pathways regulated by the p53 transcription factor and demonstrate that the p53 gene functions to maintain fidelity during cell replication. He was chairman of the department of molecular biology at Princeton University, the president of The Rockefeller University and is now professor emeritus at the Institute for Advanced Study in Princeton, NJ, USA.

Nuria López-Bigas
Institute for Research in Biomedicine,
Barcelona, Spain

Nuria Lopez-Bigas is a biologist with a PhD in molecular genetics of deafness. She transitioned into bioinformatics during her postdoc at the European Bioinformatics Institute (EBI). Since 2006, she leads a research group in Barcelona focused on the study of cancer from a genomics perspective. She is particularly interested in the identification of cancer driver mutations, genes and pathways across tumor types and in the study of their targeted opportunities. She is also interest in understanding the mutational processes leading to the accumulation of mutations in cancer cells. Her lab has done important contributions in the discovery of the variation of mutation rate along the genome. They have recently discovered that transcription factors (TF) and nucleosomes interfere with DNA damage and DNA repair producing variable mutation rate along TF binding sites and nucleosomes covered regions.

Scott W. Lowe

Scott W. Lowe, PhD
Investigator, Howard Hughes Medical Institute
Member, Memorial Sloan-Kettering Cancer Center
New York, US

Scott W. Lowe is Chair of the Cancer Biology and Genetics Program at Memorial Sloan Kettering Cancer Center (MSKCC) in New York City and an Investigator for the Howard Hughes Medical Institute. Dr. Lowe received his Bachelor's Degree from the University of Wisconsin-Madison and his Ph.D. from the Massachusetts Institute of Technology. He initiated his independent research at Cold Spring Harbor Laboratory, where his group made important contributions to our understanding of the p53 tumor suppressor network, as well as the processes of multi-step carcinogenesis, cellular senescence, and tumor-cell drug resistance. At MSKCC, his laboratory applies mouse models, functional genomics and cancer genomics in a coordinated effort to identify cancer drivers and dependencies. These efforts have revealed fundamental insights into cancer mechanisms and identified potential therapeutic targets. Dr. Lowe's work has been recognized by several awards, including a Sidney Kimmel Scholar Award, a Rita Allen Scholar Award, the Outstanding Investigator Award from the American Association for Cancer Research, the Paul Marks Prize, and the Alfred G. Knudsen Award. He has also been elected to the American Academy of Arts and Sciences and the National Academy of Sciences.

Guillermina Lozano

Guillermina (Gigi) Lozano, Ph.D.
The University of Texas MD Anderson Cancer Center
Hubert L. Olive Stringer Distinguished Chair in Oncology in Honor of Sue Gribble Stringer
Professor and Chair, Genetics Department
Member National Academy of Sciences,
Houston, Texas, US

Guillermina (Gigi) Lozano is a renowned geneticist recognized for her studies of the p53 tumor suppressor pathway. This pathway is undermined in a large percent of human cancers via mutations and deletions of p53. Her laboratory identified a transcriptional activation function for p53 (*Science* 249:1049). Using mouse models, her team characterized the physiological importance of Mdm2 and Mdm4 proteins as potent inhibitors of p53 (*Nature* 378:203; *Nature Genetics* 29:92). The Mdm proteins are over expressed in many cancers that lack p53 mutations presenting an alternate mechanism of eliminating p53 activity. Other mouse models inheriting the most common p53 mutations revealed additional gain-of-function phenotypes that drive metastases (*Cell* 119:861; *Nat Comm* 9:3953).

Dr. Lozano received her BS degree in Biology and Mathematics, *Magna Cum Laude*, at the University of Texas Rio Grande Valley. She completed graduate studies at Rutgers University and the University of Medicine and Dentistry of New Jersey, and a post-doctoral fellowship with Dr. Arnold Levine at Princeton University.

Dr. Lozano was hired as an Instructor at The University of Texas MD Anderson Cancer Center in 1987 and quickly rose through the ranks to her current position as professor and chair of the department of Genetics. Dr. Lozano is a member of the National Academy of Sciences, and the National Academy of Medicine and has received the Minorities in Cancer Research Jane Cooke Wright Lectureship, and Women in Cancer Research Charlotte Friend Lectureship awards both from the American Association for Cancer Research. She is also the recipient of distinguished alumni awards from both her undergraduate and graduate *alma maters*.

Marta Łuksza
Icahn School of Medicine, Mount Sinai,
New York, US

Marta Łuksza is an Assistant Professor at the Icahn School of Medicine, Mount Sinai, New York. She completed her Ph.D. in Computer Science at the Freie Universität and the Max Planck Institute for Molecular Genetics in Berlin. Before joining as a faculty at Mount Sinai, she was a Janssen fellow at the Institute for Advanced Study in Princeton. Her research interests are to understand biophysical mechanisms underlying the evolution of fast-adapting populations, such as pathogens and cancer cells, and to harvest these mechanisms for predictive analysis. She applies and develops methods from machine learning, statistical physics, and information theory to address these questions. She has developed models that successfully predict the dominating influenza strains in the following season, which are currently used to advise the World Health Organization with influenza vaccine selection. Her recent research focuses on predictive models for cancer evolution under immunotherapy.

Solip Park

Computacional Cancer Genomics Group Leader,
Spanish National Cancer Research Centre,
Madrid, Spain

Solip Park obtained PhD in 2012 from the Pohang University of Science and Technology in South Korea where she worked in understanding the molecular connections of phenotypically connected diseases. After obtaining the PhD she joined the Lehner's lab at the CRG in Barcelona to work on somatic and germline mutations effect to cancer. During the 6 years that she spent at the CRG to study cancer-type specific epistatic interactions and identify cancer predisposition genes through large-scale exome sequencing data. She has recently joined CNIO that focuses on the study of the cancer fitness landscape in each cancer genes across cancer types for the precision medicine.

David Posada
Biomedical Research Center,
School of Biology, University of Vigo,
Vigo, Spain

David Posada (ORCID 0000-0003-1407-3406) is an evolutionary biologist working in the Biomedical Research Center at the University of Vigo, Spain, where he is also Professor of Genetics. He was born in Spain, where he obtained his degree in Biology. He did his PhD in Zoology at BYU (Utah, USA), and his postdoc in statistical genetics in Variagenics Inc (Cambridge, USA). He is interested on theoretical, methodological and empirical aspects of the evolutionary analysis of genes and genomes. His research has been essentially cross-disciplinary, combining concepts and tools from biology, statistics and computer science. He has worked mainly on the selection of DNA substitution models, detection of recombination, coalescent simulation, phylogeography and estimation of species phylogenies. More recently, he has initiated a new but central research avenue in the field of cancer evolution, focusing on understanding the complex population dynamics of tumors over time and space, using methods adapted from organismal evolutionary biology.

Carol Prives

Columbia University,
New York, US

Carol Prives is the DaCosta Professor of Biological Sciences at Columbia University. She was educated in Canada, receiving her BSc and PhD from McGill University. Her postdoctoral training took place at Albert Einstein College of Medicine and the Weizmann Institute, after which she became a faculty member at the Weizmann Institute. She then joined the Biological Sciences Department at Columbia University where she was named the DaCosta Professor of Biology in 1995. Dr Prives served as Chair of that department between 2000 and 2004.

Since the late 1980's her work has focused on the p53 tumor suppressor protein, the product of the most frequently mutated gene in human cancers. She and her group have elucidated aspects of the structure and function of the p53 protein especially as it relates to its roles as a transcriptional activator. In parallel, her group has examined how cancer related mutant forms of p53 regulate tumorigenesis. Work from her laboratory has also illuminated the functions of the key p53 negative regulators, Mdm2 and MdmX.

Dr Prives has served as Chair of both the Experimental Virology and the Cell and Molecular Pathology Study Sections of the NIH and was a member of the NCI Intramural Scientific Advisory Board. She was also a member of the Advisory Boards of the Dana-Farber Cancer Center, the Memorial Sloan Kettering Cancer Center and the Massachusetts General Cancer Center as well as the American Association for Cancer Research. She is currently a member of the Scientific Council of the Cancer Prevention and Research Institute of Texas and is Co-Chair of the Scientific Academic Advisory Council of the Weizmann Institute of Science. She also serves on the Editorial Boards of *Cell, Genes & Development, Cancer Discovery* and the *Proceedings of the National Academy of Sciences*. Dr Prives has received several honors including being named an American Cancer Society Research Professor, election to the American Academy of Arts and Sciences, the National Academy of Medicine, the National Academy of Sciences and the AACR Academy. She has presented numerous named lectures and has received awards including the NCI Rosalind E Franklin Award for Women in Science, the Paul Jansen Prize in Advanced Biotechnology and Medicine, the AACR-Women in Cancer Research Charlotte Friend Memorial Lectureship Award and the Ernst W Bertner Award from MD Anderson. Dr Prives has also received an honorary doctorate from McGill University, her alma mater.

Raul Rabadan

Raul Rabadan Ph.D.
Columbia Systems Biology,
Columbia University,
US

Raul Rabadan is currently a tenured Professor at Columbia University with a joint appointment in the Department of Systems Biology and the Department of Biomedical Informatics, and the director of the Program for Mathematical Genomics at Columbia University and the Columbia University Center for Topology of Cancer Evolution and Heterogeneity, a NCI Physics and Oncology Center. He leads an interdisciplinary lab including mathematical, physics, computer science, engineering, and medical researchers aiming to solve biomedical problems through quantitative computational models.

Benjamin J. Raphael
Professor of Computer Science,
Department of Computer Science,
Lewis-Sigler Institute for Integrative Genomics,
Princeton University,
Princeton, US

Ben Raphael is a Professor of Computer Science at Princeton University. His research focuses on the design and application of novel algorithms for the interpretation of biological data. Recent areas of emphasis include cancer evolution, network/pathway analysis of genetic variants, and structural variation in human and cancer genomes. His group's algorithms have been used in multiple projects from The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC). He is the recipient of the Alfred P. Sloan Research Fellowship, the NSF CAREER award, and a Career Award at the Scientific Interface from the Burroughs Wellcome Fund.

Darryl Shibata

Darryl Shibata, M.D.
Department of Pathology,
University of Southern California Keck School of Medicine,
Los Angeles, CA, US

Dr. Shibata is a Professor of Pathology at the University of Southern California Keck School of Medicine in Los Angeles, CA. He is interested in reconstructing the evolution of normal and neoplastic tissues from their genomes.

Andrea Sottoriva
Deputy Director of the ICR's Centre for Evolution and Cancer,
The Institute of Cancer Research,
London, UK

Andrea Sottoriva uses genomics and computational approaches to understand cancer as a complex system. He obtained a degree in computer science and worked in particle physics before switching to biomedical research. He is the Deputy Director of the ICR's Centre for Evolution and Cancer.

Doug J. Winton

Douglas J Winton, FMedSci
Cancer Research UK Cambridge Institute,
Cambridge, UK

Following a PhD from Bristol University Doug Winton's next appointment was in the lab of Bruce Ponder at the Institute of Cancer Research in London. The focus was on analysing the mosaicism of aggregation chimaeras to infer the clonal territories created during development and how these related to epithelial tissue architecture. Subsequently, he developed mutation induced clonal marks that allowed the stem cell organisation of adult intestinal stem cells. Through the 90s and on moving to Cambridge he worked on developing inducible recombinase models for the genetic manipulation of murine epithelial tissues. Eventually this initiative created a sophisticated toolkit that allowed the detailed cell dynamics of tissue stem cells to be investigated, ultimately allowing precise inferences of stem cell number and replacement rates. Other interests are in stem cell renewal in models of both inflammation and cancer. Currently he is a Senior Group Leader at the CRUK Cambridge Institute.

Madrid 23 — 25 Sept 2019

Heterogeneity and Evolution in Cancer

Poster Session

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1

Universality of Random Matrix Theory to study single-cell sytems

Luis Aparicio[1], Mykola Bordyuh[1], Andrew J. Blumberg[2] and Raul Rabadan[1]

[1]Department of Systems Biology, Columbia University, New York, US
[2]Department of Mathematics, University of Texas, Austin, US

The development of single-cell technologies provides the opportunity to identify new cellular states and reconstruct novel cell-to-cell relationships. Applications range from understanding the transcriptional and epigenetic processes involved in metazoan development to characterizing distinct cells types in heterogeneous populations like cancers or immune cells. However, analysis of the data is impeded by its unknown intrinsic biological and technical variability together with its sparseness; these factors complicate the identification of true biological signals amidst artifact and noise. Here we show that, across technologies, roughly 95% of the eigenvalues derived from each single-cell data set can be described by universal distributions predicted by Random Matrix Theory. Interestingly, 5% of the spectrum shows deviations from these distributions and present a phenomenon known as eigenvector localization, where information tightly concentrates in groups of cells. Some of the localized eigenvectors reflect underlying biological signal, and some are simply a consequence of the sparsity of single cell data; roughly 98% is artifactual. Based on the universal distributions and a technique for detecting sparsity induced localization, we present a strategy to identify the residual 2% of directions that encode biological information and thereby denoise single-cell data. We demonstrate the effectiveness of this approach by comparing with alternative single-cell data analysis techniques in a variety of examples with marked cell populations.

Clonal competition assays to understand progression and resistance in multiple myeloma

Umair Munawar[1], Larissa Haertle[1], Lucia Martin[2], Isabel Cuenca[2], Cornelia Vogt[1], Matteo Da-Vía[1], Andoni Garitano-Trojaola[1], Leo Rasche[1], Miguel Gallardo[2], Thorsten Sthümer[1], Joaquin Martinez Lopez[2], Martin Kortüm[1] and **Santiago Barrio[1,2]**

[1]Department of Internal medicine II, Wuerzburg University Hospital, Würzburg, Germany
[2]Department of Hematology, Doce de Octubre University Hospital, CNIO, Madrid, Sapin

Progression and relapse in Multiple Myeloma (MM) is induced by changes in the clonal composition. we have developed clonal competition models based on the coculture of isogenic MM cells marked by fluorescence. To understand the effect of TP53 mono- and bi-allelic lesions, we used the TP53 wild type (WT) AMO1 cell line. After modification with CRISPR / CAS9, we selected subclones with mono- or bi-allelic deletion of TP53. For the characterization of alterations in RAS, we selected OPM2 cells, one of the few myeloma cell lines with the RAS route intact. On them, we generate the sub lines KRAS WT, G12A and A146T through stable transfection with Sleeping Beauty vectors. To study mutations related to drug resistance to commonly used drugs in myeloma we mutations in the target genes IKZF1 (WT, A152T, Q170D or R439H), CUL4B (KO), and PSMB5 (WT or A20T). All WT and mutant sublines were also stably transformed with E-GFP or LSS mKate-RFP for analysis by flow cytometry. First, we verified how lesions in TP53, both mono- and bi-allelic, induce a growth advantage in the affected cells. However, the combination of mono-allelic cells with bi-allelic favoured the latter. This "proliferative fitness" effect, was confirmed in the KRAS mutants (G12A or A146T vs. WT). Next, we characterize the effects of resistance mutations and drug exposure. IKZF1 A152T and CUL4B KO were selected against WT cells in the presence of Lenalidomide. The same effect was observed for the mutant PSMB5 A20T exposed to Bortezomib. In addition, both mutants CUL4B KO and PSMB5 A20T were overcome by WT cells when the drug was eliminated from co-culture, suggesting that these lesions only induce advantage in the presence of drug. This could explain why such alterations are not detected more frequently in relapse. Through these studies we have defined three clonal selection mechanisms; Negative Survival Fitness, induced by exposure to the drug but with a negative impact if it is eliminated (CUL4B and PSMB5). Neutral Survival Fitness (IKZF1), and "Proliferative Fitness", independent treatment exposure (mono and bi-allelic lesions TP53 and KRAS). These results shows that clonal dynamics can be characterized and quantified, and that the early detection of the lesions that govern them can be a great advantage when defining the prognosis and the best therapeutic options for MM patients.

3

Aminopyrazolonaphthoquinones: novel small molecules as a potential anti-cancer drugs

Wioletta Brankiewicz[1], Macin Serocki[1], Sławomir Milewski[1], Maciej Bagiński[1], Nataliya Marintsova[2], Nataliia Polish[2], Volodymyr Novikov[2]

[1]Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdansk University of Technology, Poland
[2]Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology, Lviv Polytechnic National University, Ukraine

One of the major challenges for cancer treatment is its heterogeneous nature, which determines the therapeutic options. Treatment strategies using small molecule compounds are one of the main trends in the study of new compounds with anticancer activity. Their small size allows them to pass through the plasma membrane and target many different internal targets such as proteins or DNA. In our study we used PNV5, PNV7, PNV9 and PNV18 (aminopyrazolonaphthoquinones) - a novel syntetic small molecules. In our work we studied the anti-cancer role of this compounds. Firstly, we evaluated the effects of PNV5, PNV7, PNV9 and PNV18 on the proliferation, apoptosis, cell cycle and ROS level. Studies were performed on different human breast cancer cells line: MDA-MB-231 (estrogen-negative breast carcinoma cells), MCF-7 (estrogen-positive receptor breast carcinoma cells), SK-BR-3 (breast adenocarcinoma cell line), BT-474 (estrogen-positive, progesterone-positive epithelial cancer cells) and non cancerous cells line (MCF-10A). The results show that these compounds are potential inducers of DNA double strand brakes and this is following by the disruption of cell cycle progression and antiproliferative effect caused in studied cells.

Genetic simulation with frequency-dependent fitness to examine the role of genetic constraints, mutator genes, and population size on cancer cooperation, competition and niche construction, and their effect on tumour progression

Ramón Díaz-Uriarte and Sergio Sanchez-Carrillo

[1]Dept. Biochemistry, Universidad Autónoma de Madrid and
[2]Instituto de Investigaciones Biomédicas "Alberto Sols" (UAM-CSIC), Madrid, Spain

Cellular cooperation and competition and niche construction are crucial for tumour development. Their consequences, and how to leverage them for treatment, have been explored by models that use game theory and adaptive dynamics. These models are often limited to a few distinct genotypes and their deterministic dynamics analysed under large population sizes. Moreover, genotypes are assumed to be mutational neighbours, and genetic constraints are rarely considered; as a consequence, these models disregard restrictions in the order of accumulation of mutations (due to epistasis), which are known to constraint the paths of tumour progression. Finally, most models do not account for mutator genes. Ignoring these factors (population size, genetic constraints, epistasis and synthetic lethality, mutator genes) is necessary for analytic tractability, but to examine complex scenarios of cooperation we need software that incorporates these phenomena.

We have developed software to simulate cooperation and competition by specifying fitness of genotypes as arbitrary functions of the frequency of any other genotypes.

Using the ExprTk library (http://www.partow.net/programming/exprtk/), we extend OncoSimulR, an R (and C++) BioConductor package for fast forward-time genetic simulation of biallelic loci in asexual populations. This gives us the capability to simulate the effects of different population sizes, mutation rates, mutator genes, and interactions between genes.

The software is available from GitHub (https://github.com/rdiaz02/OncoSimul/tree/freq-dep-fitness). We show examples to illustrate the flexibility of the software, including population collapse from frequency-dependent dynamics, and joint effects from cooperation and cytotoxic interactions.

The ability to simulate complex scenarios that involve frequency-dependent fitness should prove useful to examine the role and therapeutic use of cooperation, competition, and niche construction in cancer.

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DLBLC genetic subtypes are associated with response to therapy and overall survival

Ismael Fernández-Miranda[11], Pedrosa L[1], Pérez-Callejo D[1], González-Rincón J[1,11], Quero C[2], Rodríguez M[3,11], Tarin C[1], Martín-Acosta P[1,11], Gómez S[1], Rueda A[2], García JF[4,11], Camacho F[I,5], García-Cosío M[6], Heredero A[1], Alonso R[3], Llanos M[7], García-Arroyo FR[8], Mollejo M[9,11], Rodriguez-Abreu D[10], Piris-Villaespesa M[6], Gómez-Codina J[10], Diaz-Tejeiro C[1], Sánchez A[1], Piris MA[3,11], Provencio M[1] and Sánchez-Beato M[1]

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Diffuse large B-cell-lymphoma (DLBCL) is a heterogeneous disease in genetic alterations and clinical behaviour, and its prognosis has been associated with clinical features, cell-of-origin and genetic aberrations. The aim of the study is to determine whether somatic mutations or genes clustered in pathways are associated with overall survival (OS) and relapse (R/R) in R-CHOP treated-DLBCL patients, and whether these alterations could be integrated to better predict response to therapy.

We have analysed 67 DLBCL patients treated with R-CHOP by massive sequencing, using a custom targeted panel with 125 genes frequently mutated in lymphomas, in diagnostic biopsy samples. We performed a Schmitz/Chapuy-like classification in MCD-C5, BN2-C1 and EZB-C3 groups based on mutational data. 27 patients were defined as R/R if relapsed before 24m of treatment. Multivariate and univariate logistic regression and Kaplan-Meier method were performed for OS and R/R. The most frequently mutated genes were IGLL5 (40.3%), KMT2D (29.9%), CREBBP (28.4%), CARD11 (23.9%) and PIM1 (22.4%). Mutations in ETS1 and BCL10 were associated with higher risk of R/R after treatment ($p<0.05$), whereas patients with mutations in CD58, STAT3, CD79B, TNFAIP3, PIM1 or ETS1 had significantly shorter OS. We also analysed the impact of gene mutations in functional pathways, and we found that B-cell development were significantly associated with higher risk to R/R, while MAPK-ERK, BCR-PI3K, NF-kB, TOLL and ABC-DLBCL pathways were significantly associated with poorer OS. Analysis using the Schmitz/Chapuylike groups showed MCD-C5 and BN2-C1 had significantly shorter OS; the BN2-C1 was associated with higher risk of R/R.

The genetic groups defined by Schmitz and Chapuy are associated with OS and risk of R/R in R-CHOP treated DLBCL cases. Genetic markers, combined with clinical and molecular features, should eventually help to develop improved risk models for DLBCL patients treated with standard therapy.

High-resolution HLA typing from RNAseq and applications to cancer

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The human leukocyte antigen (HLA) complex, necessary for antigen presentation, regulates both innate and adaptive immune responses. The HLA locus therefore plays a critical role in a plethora of diseases, particularly in cancer and response to therapy. While recent improvements in the quality and accessibility of nextgeneration sequencing have made HLA typing from standard short-read data practical, this task remains challenging given the high level of polymorphism and homology between the HLA genes.

Here, we present arcasHLA: a fast and accurate in silico method to infer HLA genotypes from RNA sequencing data. Our tool outperforms the established HLA typing tools on the gold-standard benchmark dataset, with 100% accuracy at twofield resolution for HLA class I genes, and over 99.7% accuracy for HLA class II. We also present several applications on the detection of HLA allelic expression imbalance in cancer. (See Bioinformatics: doi:10.1093/bioinformatics/btz474, and code: https://github.com/RabadanLab/arcasHLA).

See Bioinformatics: doi:10.1093/bioinformatics/btz474, and code: https://github.com/RabadanLab/arcasHLA

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Quantification of protein expression to evaluate cancer heterogeneity

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Inter-tumor heterogeneity is linked to drug resistance and is thus critical to converge on treatment strategy. Thus, the diagnostic method on which patient treatment is based must accurately reflect this heterogeneity. Yet, the quantification of heterogeneity in cancer is still performed almost exclusively in research settings owing to the complexity of the methods used. We propose a workflow to quantify protein expression on a "local" level on tumor tissue sections providing a measure to evaluate heterogeneity on breast tumors.

In this workflow, we extract region of interests (ROIs) of ~150 μm in diameter from a frozen tissue section using a microfluidic probe (MFP). MFP is a liquid scanning probe device that confines a biochemical on a selected region on a tissue, with the capacity of working in liquid environments. The MFP allows the quick extraction (1.5 to 3 min) of these individual regions, termed footprints. The extracted proteins are biotinylated and analyzed through an antibody microarray. This microarray consists of a panel of 15 proteins that aids a proteomic-based differentiation of the molecular subtypes of breast cancer, i.e. luminal A, luminal B, basal and HER2. The expression of each protein is then quantified based on the greyscale intensity of the corresponding spot in the microarray and can be compared within the same tissue section to other ROIs. The rapid extraction together with the liquid environment in which the tissue is present are key for minimizing protein degradation.

Using this methodology, we observed absence of estrogen receptor (ER)=6.7 arbitrary units (a.u.) and cytokeratin 7 (CK7)=0 a.u., markers that were present in a neighboring tumor region (ER=26.4 a.u., CK7=9.10 a.u.), at a distance of ~500 μm. These differences, however, were masked when a whole tissue analysis using a microarray was performed (ER=43 a.u., CK7=0 a.u.). Hence, with this method the local extraction of tissue can help our understanding of tissue heterogeneity.

In silico prescription of anticancer drugs in single-cell RNA-seq

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The heterogeneity of cancer cells can introduce significant challenges in the design of effective treatment strategies. Since tumours can exhibit different sensitivities to cytotoxic drugs among their different clonal populations, targeted treatments could play a role in the appearance of drug resistance mechanisms. Therefore, in order to connect patient-specific data to drug response, clone-specific vulnerabilities must be taken into account.

In recent years, there have been a lot of technological advances in the development of single-cell RNA-seq (scRNA-seq) for the understanding of cell-specific transcriptome profiles. With this technology, researchers are provided with the ability to measure gene expression from individual cells, distinguishing distinct cell subpopulations, and allowing the characterization of cell proportions and composition in disease relevant tissues.

In this work, we present a novel tool for the analysis of scRNA-seq data and in silico prescription of anticancer drugs. For this purpose, we have analysed public single-cell RNA-seq tissue studies coming from the Single Cell Data Portal [1] and generated a subpopulation clustering based on genetic and chemical perturbation signatures by using the available pharmacological profiling data coming from LINCS [2] and Connectivity Map [3]. Treatments have been prioritized depending on their reach or capacity to affect a wider spectrum of the found subclones. Finally, a functional characterization of the distinct pathway mechanisms involved has been applied.

We believe this tool will lead to a better understanding of the biological and therapeutic impact of tumour heterogeneity and could be a valuable resource for personalized medicine.

[1] https://portals.broadinstitute.org/single_cell; [2] Subramanian et al. Cell, 171(6):1437-1452.; [3] https://www.broadinstitute.org/connectivity-map-cmap

9 Targeting intratumoral heterogeneity with in silico drug prescription tools

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Precision medicine is an emergent field whose aim is to develop improved prognostic, diagnostic and therapeutic strategies for each patient according to thousands of people's clinical and genomics data. Precision medicine is particularly interesting in oncology, since cancers are characterized by a high genomic heterogeneity among different tumors (intertumoral heterogeneity) and within the same cancer (intratumoral heterogeneity, ITH). Recently, some in silico drug prescription tools have emerged to prioritize tumor's specific genomic alterations with matched therapies and candidate drugs. Nevertheless, these resources often use a therapy administration strategy based on bulk results and ignore ITH.

ITH refers to the existence of different cell populations within the same tumor and can be spatial (variability in different locations) or temporal (variability over the temporal evolution of the tumor). ITH has been revealed as a key factor in cancer patients' outcome contributing to higher lethality, therapy failure and drug resistance. Thus, in silico drug prescription performance could be improved by integrating ITH information into preexistent tools.

In this work, we used PanDrugs (www.pandrugs.org), an in silico drug prescription tool developed in our laboratory, to design anticancer treatment regimens considering temporal and spatial ITH. PanDrugs is a bioinformatics platform that identifies druggable genomic alterations and prioritizes drug therapies based on clinical, biological and pharmacological evidence. Our results indicate that it is possible to identify drugs or combinations capable of covering the clonal diversity of the tumor. This strategy can help to target minority clones that would otherwise be favored by the administration of the treatment and cause relapse. Moreover, we show that ITH dissection can be a very valuable strategy to increase the therapeutic options in cancer patients.

This study applied PanDrugs to mutational profiles obtained by temporal and spatial ITH dissection in acute myeloid leukaemia (AML) [1] and non-small cell lung cancer (NSCLC) patients [2] respectively.

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Subclonal distribution of somatic copy number alterations determines recurrence in early-stage colon cancer

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Human cancers are complex diseases that genetically change over time. Chromosome instability leads to the co-existence of distinct subclonal cell populations, resulting in intratumor heterogeneity (ITH), which has already been associated with poor clinical outcome in some cancer types. Here, we performed SNP-arrays in 92 stage II (pT3-T4, N0) colon carcinomas, including 39 patients showing recurrence after a 5-year follow-up and 53 without. None of the patients received adjuvant treatment. Also, we performed fluorescence in situ hybridization (FISH) to examine the dynamics of copy number alterations (CNAs). Finally, levels of immune infiltration were assessed by immunohistochemistry analysis of CD3+ and CD8+ cells. Our data showed that tumors from patients with recurrence had a larger fraction of aneuploid genome. Candidate genomic regions displaying higher prevalence in recurrent tumors were the gain of chromosome 13q, loss of 17q21.31-q24.3 and copy-neutral LOH at 17p13.3-p11.1. Levels of ITH were assessed by measuring the proportion of subclonal CNAs, defined as those being present in <85% of the tumor population. Given the positive correlation between the percentage of aneuploid genome and the presence of ITH ($R=0.6$, $P<0.0001$), we further interrogated the levels of ITH as a potential biomarker of recurrence. The results indicated that tumors from patients with recurrence showed a greater proportion of subclonal CNAs compared to tumors from patients without recurrence ($P=0.04$). The analysis of the distribution of subclonal populations carrying the gain of chromosome 13 by FISH confirmed that recurrent tumors presented higher amounts of ITH ($P<0.0001$). Finally, tumors with high CNA loads displayed reduced levels of activated infiltrating lymphocytes ($P=0.02$). In summary, these data suggest that ITH is associated with an increased risk of disease relapse in early-stage colon cancer, a finding that brings up ITH in the spotlight of clinical decision making.

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Tumor Heterogeneity in advanced pancreatic ductal adenocarcinomas upon combined inhibition of EGFR and c-RAF

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancerrelated deaths, with a five-year survival rate around 5–7%. PDAC belongs to one of the most chemoresistant cancers, due to the broad heterogeneity of genetic mutations and dense stromal environment. Our recent results illustrate that in our K-Ras/Trp53 driven PDAC mouse model, PDACs are composed of at least two distinct tumor cell populations, based on their different response to the elimination of EGFR and c-Raf and their transcriptional profile (1). Importantly, the two populations correlate with two previously described clinical subtypes of human PDAC: basal, that has the worst prognosis, and classical. We are currently studying the origin of this tumor heterogeneity, since we know it is not driven by acquired mutations in other oncogenes during tumor progression or due to the loss of tumor suppressor genes. However, these two populations cannot be distinguished at a histological level by H&E staining. RNA sequencing and phosphoproteomic analysis of isolated pure populations generated from different tumors by single-cell subcloning will help to define the nature of this heterogeneity that interferes with the efficacy of our therapeutic strategy.

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Adenomatous diversification precedes malignant transformation during transition from colorectal adenoma to carcinoma

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Although colorectal carcinoma (CRC) development is well accepted as a stepwise and sequential process, it remains commonly investigated using non-sequential or non-paired adenoma and carcinoma samples. In our current study, we compare the molecular pathology of the earliest carcinoma with its matched adenomatous precursor, found side by side in a single sample. In this manner we unravel common and divergent genetic traits between matched neoplasms with a shared evolutionary history but different contemporary pathology. We compared mutations and copy number alterations between regionally dissected tissue using panel and whole-exome sequencing from 34 polypectomies or earlystage resections. Immunohistochemistry was employed to detect TP53 expression patterns, while DNA copy numbers were validated by fluorescence in situ hybridization. In addition, we conducted multi-regional sampling of histologically distinct regions within samples and sequenced them at ultra-high depth. Our results identified TP53 alterations, chromosome 20q copy number gains and microsatellite instability as the earliest drivers of malignant CRC transformation. We find that despite similar total number of overall mutations between matched adenoma and carcinoma neoplasms, they each harbored private (distinct) driver mutations and recurring copy number changes. We identified exclusive adenomatous component mutations in PIKCA and KRAS. When key alterations in TP53 mutations and chromosome 20q copy number gains happened to be shared between matched samples they were mainly in adenomas with high-grade histology. This indicates high-grade adenomas already harbor alterations indicative of cancer transition although they have not yet morphologically transitioned to cancer. Clonal evolutionary analysis into samples revealed complex early mixing and non-linear trajectories of transition. Our results demonstrate that driver alterations arise earlier in CRC progression than previously considered.

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Insight into the taxonomic and functional profiles of microbiome associated with cervical cancer development

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Cervical microbiome is associated with cervical cancer risk, but microbialcompositional and functional profile change in cervical intraepithelial neoplasia process remains unclear. Herein, we investigated microbial-compositional and functional changes between control group and CIN2-3 and cancer (diseased) group. We carried out 16S rRNA amplicon sequencing on 50 and 42 samples from control and diseased groups, respectively. PICRUST program from EzBioCloud pipeline was applied to identify the taxonomic and functional biomarkers associated with cancer. Mann-Whitney was used for measuring the statistical significance of α-diversity and β-diversity. Linear discrimination analysis effect size (LEfSe) was carried out to assess the enrichment in assigned taxonomic and functional profiles. Subsequently, we found differentially taxa and pathways and orthologies that are characteristic of cervical cancer. Overall, we demonstrate a lower richness in control group as compared with diseased group; however, the β-diversity tended to be similar in the groups. We observed that selected LAB were increased in control group relative to diseased, except for L. iners, but none of them reached a statistical significance. LEfSe demonstrated that the species, including Prevotella amnii, Fusobacterium nucleatum, Enhydrobacter aerosaccus, Corynebacterium striatum, Micrococcus luteus, Streptococcus pneumoniae, Cutibacterium acnes, Massilia alkalitolerans, Weissella koreensis and nine others uncharacterized species were characteristic for cervical carcinogenesis. Our finding demonstrated metabolic change in cervical epithelial during carcinogenesis. Specially, two pathways, folate biosynthesis and oxidative phosphorylation, which meet cell proliferation need during cervical carcinogenesis. Therefore, our findings may provide novel biomarkers enabling the early cancer detection.

Charles N. Tango has completed his PhD from Kangwon National University and is working now as Research Assistant at National Cancer Center, Republic of Korea. His research is focused on Microbiology, Oncology, and Biology Molecular.
Currently his working on metagenomic and metabolomics study using 16S rRNA or shotgun sequencing and bioinformatics tools for identifying the taxonomic and functional role of microbiome that are associated with carcinogenesis.

TCF4 regulates melanoma phenotype switching and drives therapy resistance

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The genetic events underlying cancer initiation and progression have been mostly deciphered through large sequencing efforts of cancer genomes. However, it has become clear that our understanding of the molecular events driving this disease remains limited as overwhelming evidence indicates that also nongenetic mechanisms contribute to tumorigenesis. Indeed, melanoma is characterized by a phenomenon called "phenotype switching", a transcriptional reprogramming event – genetically independent – that endows cells with specific phenotypic properties through cues of the microenvironment or therapeutic stress. Melanoma cells in a more mesenchymal state, compared to melanocytic state, have increased drug resistance, invasion capacity and are the most probable culprit for relapse. Therefore, understanding the phenotype switching mechanism and how to target the mesenchymal resistant cancer cells is pivotal.

Here we show that TCF4, a transcription factor already linked to epithelialmesenchymal transition and invasion, controls the mesenchymal state and the resistant phenotype in melanoma. Our results demonstrate that TCF4 can inhibit the expression of the melanocytic signature by inhibiting its master regulator MITF whilst inducing the expression of the mesenchymal signature. Interestingly, high activity of TCF4 corresponds to increased invasion *in vitro* and drug resistance *in vivo*. We also propose a new method to indirectly target TCF4 using a BET inhibitor, showing its synergistic effect with BRAF and MEK inhibitors.

In conclusion, we propose that the transcription factor TCF4 might be a master regulator in melanoma driving disease progression and drug resistance.

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Evolutionary dynamics leads to superlinear scaling laws and explosive growth in human cancers

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Many complex entities composed of a large number of interacting individual elements obey simple laws when viewed as a function of their size, the so-called scaling laws. For instance, the Kleiber law relates body mass and metabolism for most life forms. The fact that metabolism grows as a 3/4-power of the mass is a reflection of the increasing efficiency of biological systems as they grow larger.

For human cancers one would expect the scaling to be between ¾ as in efficient living systems, and 1 for uncoordinated multicelular systems. Here we report the surprising discovery of metabolic scaling laws in human cancers that have superlinear exponents (>1). To do so we used datasets of head & neck, non-small cell lung, locally advanced breast and rectal cancer imaged with PET. The increase in metabolism with size is found to be the result of a progressive acceleration in proliferation due to evolutionary dynamics, also observed in this work in datasets of gliomas and breast cancer imaged with PET using proliferation markers (Choline and FLT respectively). The results of this study based on PET of 500 patients suggested, through mathematical modeling, that untreated cancers would have a super-exponential explosive growth. We validated this dynamic using longitudinal volumetric imaging data from 30 untreated cancer patients of different histologies (atypical meningiomas, brain metastasis, NSCLC and gliomas) selected from a dataset of more than 1,000 records imaged with high-resolution CT and/or MRI, and also in two different animal models.

Our observations were explained using biologically-inspired mechanistic mathematical models assuming an underlying evolutionary dynamics.

Finally, the scaling laws thereby identified allowed us to define a set of very accurate prognostic metrics for tumors for which both PET and survival data were available (NSCLC, gliomas, breast cancer and head and neck cancer) thus providing an added clinical value to the base findings.

Unravelling the molecular pathways involved in cancer metastasis using cell genetic tracing strategies, next-generation and single-cell sequencing technologies

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Intratumor genetic and transcriptomic heterogeneity has been observed in multiple cancers and has been postulated as a key driver for tumor metastasis and treatment resistance1,2. In contrast to the traditional view of the metastasis as a very late event in tumor progression, genetic analyses of circulating tumor cells and functional studies in animal models, have suggested that the dissemination of tumor cells can be a very early event, even at a premalignant stage3. Moreover, transcriptional analysis of cells with different metastatic potential in mouse tissues have been used to identify genes and pathways involved in metastasis specificity4. In this context, the combination of cell lineage tracing systems, next-generation and single-cell sequencing technologies in genetically modified mouse models provide new opportunities to unravel the molecular mechanisms behind metastatic potential. We have generated a new improved fluorescent-based lineage tracing system that solves some of the limitations regarding unique marker identification and the number of marker combinations showed by available systems. Using fluorescent-based lineage tracing systems in a mouse model of pancreatic cancer we have observed that advanced tumors are monofocal in origin and present intratumor heterogeneity with clones showing tissue metastatic specificity5,6,7. Additionally, we have applied new combinatorial labeling single-cell RNA-sequencing strategies to our mouse model that are very flexible and do not require any specific infrastructure. These experiments have unraveled the presence of cells with different transcriptional programs inside murine pancreatic tumors. In summary, we have generated very promising new tools that could open new opportunities to study the molecular mechanisms behind metastatic potential in pancreatic tumors. This will surely increase our molecular knowledge in cancer progression and eventually improve the treatment of the patients with this fatal disease.

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Examination of the role of PAK4 in transgenic mouse models of breast and pancreatic cancer

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P21-activated kinases (PAKs) are effectors of the small GTPases Cdc42 and Rac that have been implicated in various cancer forms. PAK4 is overexpressed in both breast and pancreatic cancer; however, the functional in vivo role PAK4 in tumor development remains unclear.

Here we explored the role of PAK4 *in vivo* in transgenic mouse models of pancreatic and breast cancer. Due to the early embryonic lethality of homozygous PAK4 knock-out in the mouse, we crossed PAK4 fl/fl mice with MMTV-Cre and Pdx-Cre promoters to generate mice carrying conditional PAK4 depletion in the epithelium of the mammary gland and pancreas and observed no defects in organ development. These mice were then crossed with MMTV-PyMT and LSL-KrasG12D mice, respectively, to create MMTV-Cre; MMTV-PyMT; PAK4 fl/fl and Pdx1-cre; LSL-KrasG12D; PAK4 fl/fl mice. Results from the MMTV-PyMT model suggest that loss of PAK4 in the mammary gland delays tumor onset in both female and male mice. Analysis of Pdx1-cre; LSL-KrasG12D; PAK4 fl/fl mice are ongoing and will be reported at the conference.

Our results suggest that conditional ablation of PAK4 impairs PyMT-driven mammary tumorigenesis and reveal a selective vulnerability of breast cancer to PAK4 inhibition.

A bi-stable transcriptional network motif governs self-renewal and differentiation in SCCs

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Basal tumor propagating cells (TPCs) control squamous cell carcinoma (SCC) growth by self-renewing and differentiating into supra-basal SCC cells, which lack proliferative potential. While transcription factors such as SOX2 and KLF4 can drive these behaviors, their molecular roles and regulatory interactions with each other have remained elusive. Here, we show that PITX1 is specifically expressed in TPCs, where it co-localizes with SOX2 and TRP63 and determines cell fate in mouse and human SCC. Combining gene targeting with chromatin immunoprecipitation sequencing (ChIP-seq) and transcriptomic analyses reveals that PITX1 cooperates with SOX2 and TRP63 to sustain an SCC-specific transcriptional feed-forward circuit that maintains TPC-renewal, while inhibiting KLF4 expression and preventing KLF4-dependent differentiation. Conversely, KLF4 represses PITX1, SOX2, and TRP63 expression to prevent TPC expansion. This bistable, multi-input network reveals a molecular framework that explains selfrenewal, aberrant differentiation, and SCC growth in mice and humans, providing clues for developing differentiation-inducing therapeutic strategies.

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Identification of novel genetic subgroups and activation mechanisms of mTOR pathway in Pancreatic Neuroendocrine Tumors (PNENs)

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Pancreatic Neuroendocrine tumors (PNENs) are a rare neuroendocrine malignancy, lacking a clear genomic characterization, which can depict the intertumor heterogeneity. To identify recurring single nucleotide aberrations (SNA) in driver genes, we established a gene panel, targeting 47 genes and performed deep coverage (1158 reads) sequencing on 58 PNEN samples. Additionally, we subjected 32 of these samples to Infinium methylation EPIC bead chip array to establish recurring copy number aberrations (CNA). We further used DigiWest proteomics platform to determined differentially aberrant signaling pathway in 15 samples Targeted panel sequencing yielded SNAs in 33 samples, and the most recurrently altered gene was MEN1 (33%). CNA data subdivided our patient cohort into two major entities: a patient group harboring recurring copy number gains in chromosomes 5, 7, 9 (and 20) (high-CNA) and a patient group with relatively low or no chromosomal aberration (low-CNA). Hierarchical clustering suggested that gain in these chromosomes might be a co-occurring event. Proteomic analysis revealed 5 candidate proteins that are differentially expressed between the groups ($p < 0.05$): RPS6, NPM, CDK1, RASSF2 and EIF4E. Hierarchical clustering of significantly different analytes showed a clear aggregation of high-CNA and low- CNA samples. Genes encoding the differentially expressed proteins belonged to chromosomes gained in the high-CNA group. Across these set of chromosomes, difference in the mean log2 protein change of all proteins under consideration showed a generally higher expression trend in the high-CNA group. Prior work from others has shown that increased expression in the differentially expressed proteins we found have an impact on the activation of the mTOR pathway. In conclusion, our work identified genomic and proteomic features that better define and subgroup PNENs and also determined a set of differentially expressed proteins which characterizes these PNEN subgroups.

Regulation of expression of genes from the Grainyhead-like family (GRHL) – new molecular insights into cancer development

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Genes from the Grainyhead-like family are found in all animal species and fungi that were studied so far. In mammals there are three genes that belong to this family: Grainyhead-like 1 (GRHL1), Grainyhead-like 2 (GRHL2) and Grainyhead-like 3 (GRHL3). The expression of these genes is tissue- and developmentally-specific, and occurs primarily in epithelia. Development of many types of cancer is often accompanied by changes in the levels of expression of the genes from the GRHL family. GRHL genes are directly involved in the process of carcinogenesis.

In order to predict transcription factors regulating the expression of GRHL genes, we conducted bioinformatic analyses of the promoter regions for each gene: GRHL1, GRHL2, and GRHL3. Subsequently, using appropriate experimental methods (chromatin immunoprecipitation, quantitative real time PCR, etc.) we verified whether the predicted transcription factors indeed regulate the expression of GRHL genes.

In our project we discovered additional transcription factors regulating the expression of GRHL genes. Consequently, our findings may allow to identify novel drug targets in signaling pathways whose activation or inhibition may lead to changes in the levels of expression of GRHL genes.

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Identification of a tumor gene expression signature of breast cancer risk: association with disease aggressiveness and survival.

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Breast cancer cases having low onset risk (as defined by Tyrer-Cuzick (TC) model scores) are more likely to develop more aggressive tumor characteristics. The aim of this study was to evaluate RNA-seq gene expression differences among primary invasive tumors explained by lower 5-year TC to better understand its relationship with disease subtypes and aggressiveness.

In a discovery validation design, a TC-related gene expression signature (TC-Gx) was computed and found associated with more aggressive PAM50 subtypes (per 1-SD increase in TC-Gx, basal-like OR: 13.21; 95% CI, 7.01 to 24.57; HER2-enriched OR: 4.79; 95% CI, 2.95 to 7.79, in the validation data set), tumor characteristics, interval cancers, and 10-year breast cancer-specific survival (HR: 2.29; 95% CI, 1.21 to 4.35, in discovery-validation). Associations with PAM50 subtypes were replicated in an external data set from The Cancer Genome Atlas. We found differential gene expression for lower TC to be significantly enriched for gene sets related to proliferation and estrogen signaling processes.

In conclusion, cases exhibiting an expression profile related to lower TC were more likely to develop more aggressive subtypes and to have worse survival. Our study contributes towards better molecular understanding of the relationship between onset risk and aggressiveness of breast cancer, implying the need to establish risk-related factors associated with more aggressive disease in order to lower disease burden.

Melanoma initiation: the melanoma cell of origin in a novel, human-like melanoma mouse model

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Cutaneous melanoma is the deadliest skin disease. It arises from melanocytes that suffer malignant transformation due to mutations, usually enhanced by UV radiation. Recently, novel therapeutic approaches have been developed, improving overall survival of patients with melanoma. Furthermore, the vast majority of these patients develop drug resistance. Therefore, it is important to characterize the melanoma cells of origin to find novel therapeutic approaches. Human melanoma arises from the melanocytes residing in the interfollicular epidermis. Understanding tumor initiation mechanisms at molecular and cellular level is crucial to identify novel therapeutic targets in order to prevent and cure melanoma. However, current mouse models do not recapitulate early events of human melanoma, emphasizing the need for an in vivo mouse model that mimics human anatomic-pathophysiological features of melanoma disease. We have developed a unique genetically engineered melanoma mouse model on a Tabby background. The Tabby phenotype is caused by a mutation in a mouse orthologue of the EDA gene, which presents skin and hair abnormalities like absence of hair follicles in the tail. Therefore, we suggest that this particular melanoma mouse model is ideal to study the different stages of human melanoma progression. Using the TyrCreERT2/LoxP system in the Tabby mice, we induced a melanocyte-specific BrafV600E mutation, which is present in at least 50 % of human melanomas, and a deletion of the tumor suppressor Pten. In this particular mouse model, we observed that tumor initiation establishes in the interfollicular epidermis, mimicking human melanoma pathology. Using Tabby melanoma mouse model, we aim to discover specific markers of early stages of melanoma using single cell transcriptome analysis. Subsequently, we will validate the findings in human melanoma patient samples.

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