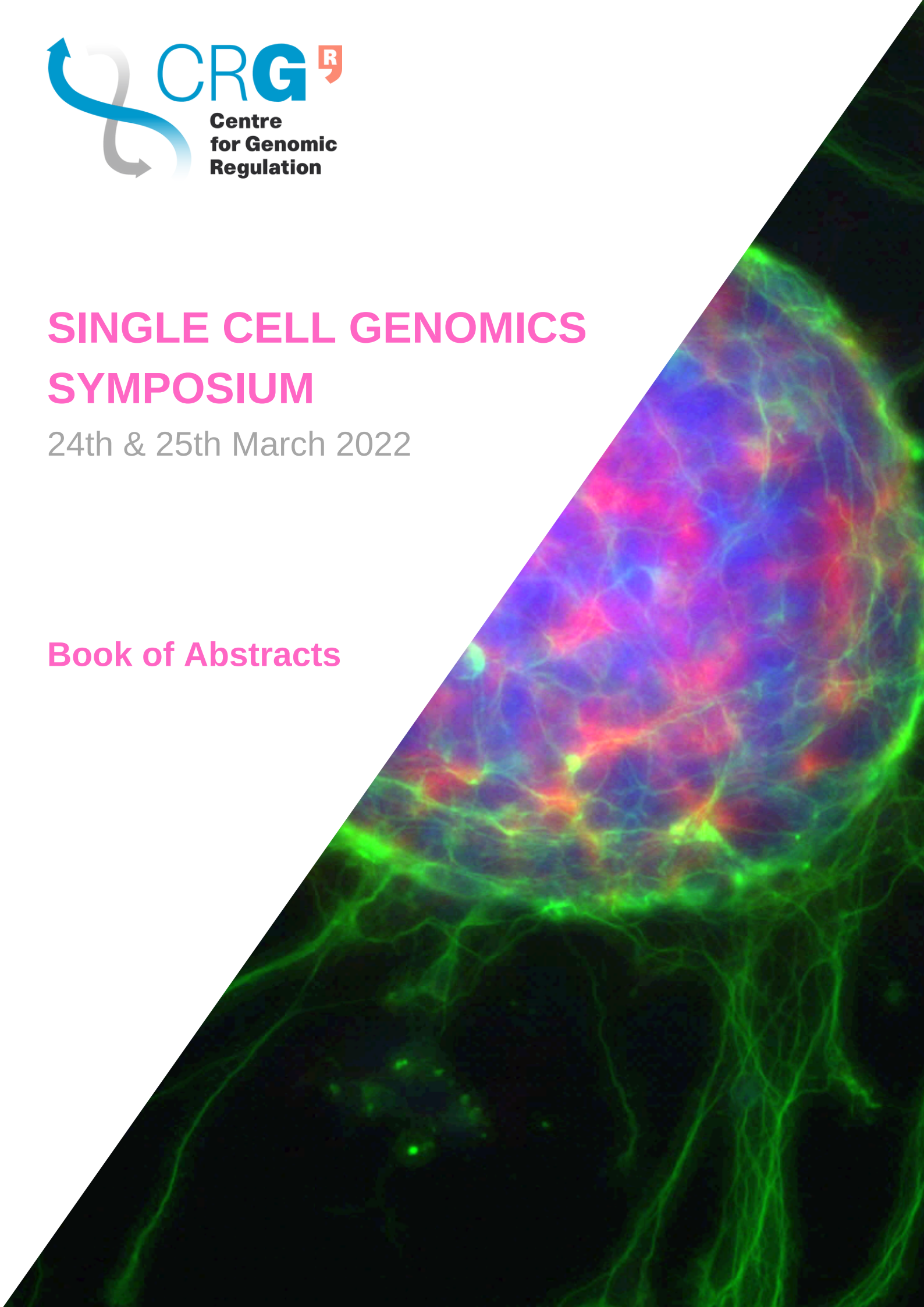
Centre for Genomic Regulation

SINGLE CELL GENOMICS SYMPOSIUM

24th & 25th March 2022

Book of Abstracts



ORGANISED BY:

CRG Centre for Genomic Regulation

IRB BARCELONA INSTITUTE FOR RESEARCH IN BIOMEDICINE

Josep Carreras LEUKAEMIA Research Institute

SPONSORED BY:

Platinum Sponsors

nanoString

DIAGNÓSTICA LONGWOOD

mission bio

BONSAILAB

10X GENOMICS

izasa scientific a werfen company

Bronze Sponsors

illumina

resolve biosciences

BD

PerkinElmer For the Better

Silver Sponsors

TECAN.

CELLENION A BICO COMPANY

Supported by

The Company of Biologists

PROGRAM

MARCH 24th

9:00-9:10	Welcome
9:10-9:45	**"New genomic technologies to deconstruct cell identity in reprogramming and development"** Samantha Morris | Washington University in St. Louis
	Session 1 - Single cell epigenomics and cancer
09:50-10:15	**"High-throughput targeted single-cell DNA methylation analysis for uncovering epigenetic heterogeneity"** Renee Beekman | Centre for Genomic Regulation (CRG)
10:15-10:40	**"Decoding HIF pathway expression and therapeutic potential in human acute myeloid leukemia-initiating cells evolution using single-cell transcriptomics"** Juan Luis Trincado (Menendez's Lab) | Josep Carreras Leukaemia Research Institute (JCI)
10:40-11:05	**"Atlasing Human Primary Immunodeficiencies"** Esteban Ballestar | Josep Carreras Leukaemia Research Institute (JCI)
11:05-11:25	**"Unleashing the Power of Single-Cell Multi-Modal Analysis to Advance Precision Medicine"** Gema Fuerte, Senior European Field Application Scientist, Missio Bio
11:25-11:55	Coffee break
	Session 2 - Single cell technology
11:55-12:20	**"TBD"** Holger Heyn | Centro Nacional de Análisis Genómico (CNAG-CRG)
12:20-12:40	**"Unsupervised discovery of sub- and intercellular patterns of expression via deep generative modeling on spatial multi-omics data"** Luca Marconato | EMBL Heidelberg (contributed talk)

12:40-13:00 **"Single-cell genome-plus-transcriptome sequencing without upfront genome preamplification reveals differential cell state plasticity and treatment response between genetic subclones"**
Sebastiaan Vanuytven | KU Leuven (contributed talk)

13:00-13:20 **"Resolving Spatial Heterogeneity Using Nanostring's High Precision and Single-Cell Profiling Platforms"**
Niccolo Mariani | Technical Sales Specialist, NanoString Technologies

13:20-15:00 Lunch

14:00-14:30 **Special Workshop: "Open Science & Publishing Tips"**
Novella Guidi | Life Science Alliance (EMBO Press, Rockefeller University Press, Cold Spring Harbor Laboratory Press)

Session 3 - Single cell perspectives on hematopoiesis

15:00-15:35 **"Democratizing single cell research"**
Thomas Ayers | Dolomite Bio | Izasa Scientific (sponsored talk)

15:35-16:00 **"Transcriptional regulation of the trophectoderm-ICM bifurcation during pre-implantation embryo development"**
Thomas Graf | Centre for Genomic Regulation (CRG)

16:00-16:20 **"Single-cell multi-omics reveal routes of purifying selection and metabolic vulnerabilities due to pathogenic mitochondrial DNA"**
Leif Ludwig | Berlin Institute of Health (contributed talk)

16:20-16:50 Coffee break

16:50-17:15 **"Single-cell lineage analysis of aged and premalignant hematopoietic stem cells"**
Alejo Rodriguez-Fraticelli | Institute for Research in Biomedicine (IRB Barcelona)

17:15-17:40 **"Clonally resolved single-cell multi-omics enables the identification of cancer specific surface antigens"**
Lars Velten | Centre for Genomic Regulation (CRG)

17:40-18:15 **"Deciphering TP53 mutant Cancer Evolution with Single-Cell Multi-Omics"**
Adam Mead | University of Oxford

18:15-20:00 Social hour and Poster session

MARCH 25th

Session 4 - Single cell genomics and human disease

09:00-9:25 **"Heterotypic cell interactions direct neoplastic competence within the pre-malignant pancreas"**
Direna Alonso | IRB Barcelona (contributed talk)

09:25-09:45 **"Developmental origins of cell heterogeneity in the human lung"**
Sergio Marco-Salas | Stockholm University (contributed talk)

09:45-10:10 **"Single-cell multi-omics reveal routes of purifying selection and metabolic vulnerabilities due to pathogenic mitochondrial DNA"**
Elisabetta Mereu | Josep Carreras Leukaemia Research Institute (JCI)

10:10-10:45 **"From genotype to phenotype with single-cell resolution"**
Oliver Stegle | German Cancer Reseach Center

10:45-11:15 Coffee break sponsored by 10xGenomics

Session 5 - Single cell genomics and fundamental principles of biology

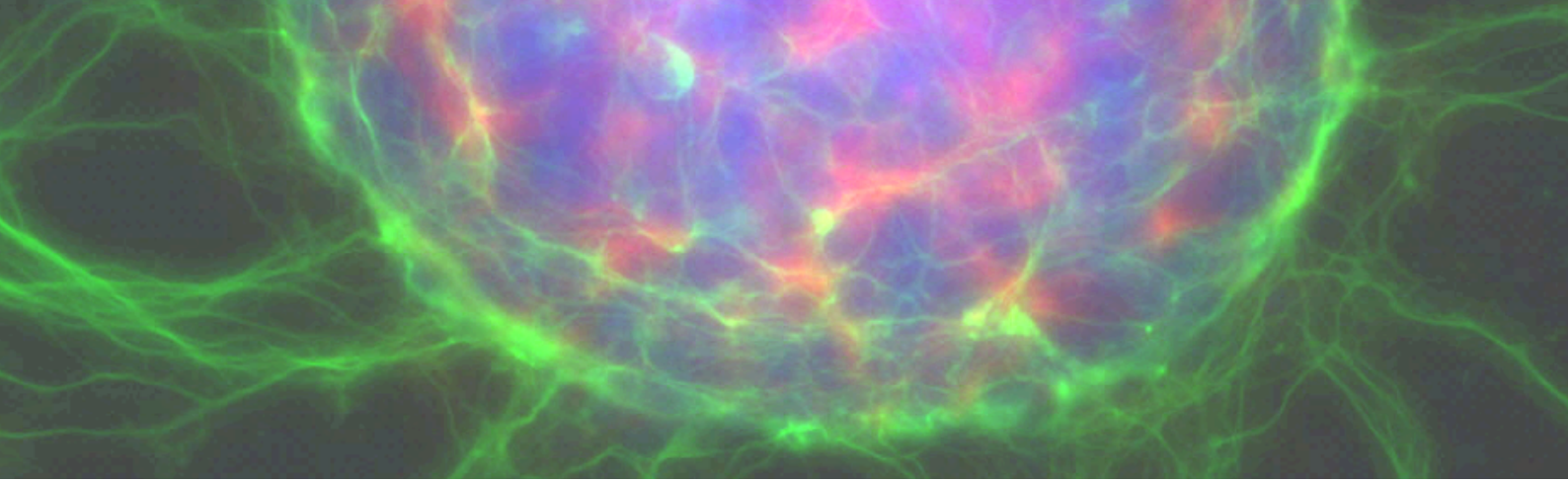
11:15-11:40	**"Single cell approaches for compartive and regulatory characterization of major animal cell types"** Anamaria Elek (Sebé-Pedrós lab) | Centre for Genomic Regulation (CRG)
11:40-12:05	**"Untangling the structure of gene regulatory networks during adaptation"** Mariona Nada-Ribelles (Posas' lab) | Institute for Research in Biomedicine (IRB Barcelona)
12:05-12:25	**"New Perspectives in Single Cell: Pushing the Boundaries with 10x Genomics Solutions"** Elisabetta Mereu | Josep Carreras Leukaemia Research InLuca Mazzitelli | 10xGenomics | Bonsai Lab (sponsored talk)
12:25-12:50	**"Single-cell transcriptomics of iPSC-derived neurons reveals functional changes in Alzheimer's Disease"** Mireya Plass or lab member | Bellvitge Biomedical Research Institute (IDIBELL)
12:50-13:10	**"Decoding the developing human immune system"** Muzz Haniffa | Newcastle University & Wellcome Sanger Institute
13:10-13:20	Closing remarks
13:20-15:00	Lunch

SAMANTHA MORRIS

Washington University in St. Louis

"New genomic technologies to deconstruct cell identity in reprogramming and development"

Direct lineage reprogramming involves the remarkable conversion of cellular identity. Single-cell technologies aid in deconstructing the considerable heterogeneity in transcriptional states that typically arise during lineage conversion. However, lineage relationships are lost during cell processing, limiting accurate trajectory reconstruction. We previously developed ‘CellTagging,’ a combinatorial cell indexing methodology, permitting the parallel capture of clonal history and cell identity, where sequential rounds of cell labeling enable the construction of multi-level lineage trees. CellTagging and longitudinal tracking of fibroblast to induced endoderm progenitor (iEP) reprogramming reveals two distinct trajectories: one leading to successfully reprogrammed cells and one leading to a dead-end state. Here, I present new methods to enable the molecular mechanisms underlying reprogramming outcomes to be dissected. First, I will cover experimental technologies to record gene regulatory events, in individual cells, in the earliest stages of reprogramming.



Second, I will introduce new computational tools to measure cell identity and reconstruct changes in gene regulatory network (GRN) configurations across the reprogramming process. Together, these tools provide new mechanistic insights into how transcription factors can drive changes in cell identity and help reveal new factors to enhance the efficiency and fidelity of reprogramming.

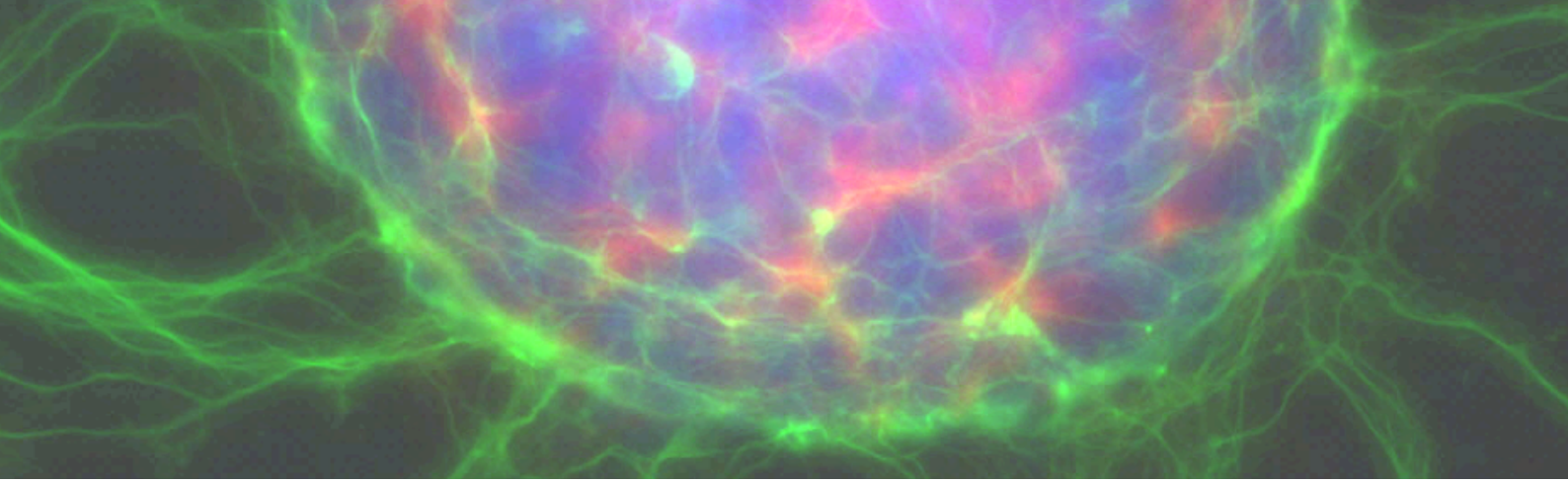
RENNÉ BEEKMAN

Centre for Genomic Regulations (CRG)

"High-throughput targeted single-cell DNA methylation analysis for uncovering epigenetic heterogeneity"

DNA methylation is an epigenetic mark extensively modulated during physiological and pathological processes. This has motivated the development of single-cell DNA methylation analysis methods to assess regulatory mechanisms and epigenetic heterogeneity in a high variety of biological contexts. Nevertheless, the large sequencing effort required due to the high number of CpGs in the genome, limits current single-cell methylome profiling methods, with available techniques producing very sparse datasets. This, together with the low cellular throughput of many existing methods, largely restrains the ability to examine locus-specific DNA methylation changes in a large number of cells at base-pair resolution.

Here we report on a high-throughput method for single-cell targeted analysis of the methylome (scTAM-seq). This technique facilitates the analysis of DNA methylation of customizable panels of approximately 500 CpGs, yielding a reliable DNA methylation readout across 5000 cells per sample on average.



We have applied scTAM-seq to study the DNA methylation profiles of B-cell specific DNA methylation sites in CD19+ B-cell subpopulations observing that our technique is capable (i) to reliably detect differential methylation patterns in B cells, (ii) to distinguish major B-cell cell populations as well as smaller subpopulations, (iii) to assess for a subset of regions if it is methylated on one or two alleles per cell, which is difficult to assess from bulk data.

In conclusion, we have developed a new single-cell method that allows to obtain reliable targeted DNA methylation read outs, which will facilitate the study of DNA methylation in a large variety of biological contexts.

JUAN LUIS TRINCADO

Centre for Genomic Regulations (CRG)

"Decoding HIF pathway expression and therapeutic potential in human acute myeloid leukemia-initiating cells evolution using single-cell transcriptomics"

Relapse represents one of the main challenges to reach the complete cure of acute myeloidleukemia (AML) patients. Biologically, relapse is driven by a group of therapy-resistant leukemiainitiating cells with stem cell properties. These cells reside in a specific hypoxic niche within thebone marrow. Hypoxia signalling keeps cells in a quiescent and low metabolic status making cellsless sensitive to cytostatic drugs used in clinical therapies. Thus, hypoxia represents a potentialtarget to sensitize leukemia stem cells (LSC) to these chemotherapeutic compounds. This studyprovides an exhaustive and comprehensive analysis of the LSC population in human samples ofspecific subtypes of AML by single cell transcriptomics, focusing on the functional and molecularcharacteristics of the hypoxia/HIF pathway. Although, we detected this pathway weakly expressedin LSCs in comparison with the more differentiated cells within the leukemia, we found that itsinhibition substantially affects the survival of LSCs in combination with AraC treatment. Thesefindings highlight the role of HIFs as stem regulator and its potential use as a therapeutic target inAML.

ESTEBAN BALLESTAR

Josep Carreras Leukaemia Research Institute (JCI)

"Atlasing Human Primary Immunodeficiencies"

Common variable immunodeficiency (CVID), the most prevalent symptomatic primary immunodeficiency, displays impaired terminal B-cell differentiation and defective antibody responses. Incomplete genetic penetrance and ample phenotypic expressivity in CVID suggest the participation of additional pathogenic mechanisms. Monozygotic (MZ) twins discordant for CVID are uniquely valuable for studying the contribution of epigenetics to the disease. For the study presented here, we generated a single-cell epigenomics and transcriptomics census of different B cell subpopulations (naïve and unswitched and switched memory B cells) in a CVID-discordant MZ twin pair. Our analysis identified DNA methylation, chromatin accessibility and transcriptional defects in memory B-cells that mirroring defective cell-cell communication upon activation. These findings were validated in a cohort of CVID patients and healthy donors. Our findings provide a comprehensive multi-omics map of alterations in naïve-to-memory B-cell transition in CVID and reveal links between the epigenome and immune cell cross-talk. Our resource, publicly available at the Human Cell Atlas, paves the way for future diagnosis and treatments of CVID patients.

Sponsored talk

GEMMA FUERTE

Missio Bio

"Unleashing the Power of Single-Cell Multi-Modal Analysis to Advance Precision Medicine"

- How is Tapestri unique to the industry
- Tapestri workflow overview
- Tapestri capabilities on SNV, CNV, and protein sequencing at scale
- Tapestri applications in oncology and cell and gene therapy

Contributed talk

LUCA MARCONATO

EMBL Heidelberg

"Unsupervised discovery of sub- and intercellular patterns of expression via deep generative modeling on spatial multi-omics data"

Assays for spatial molecular profiling which enable the localization of expression within the tissue, are now widely accessible and make possible the exploration of biological patterns at the inter-cellular, and even subcellular level. As for conventionals cRNA-seq data, the measurements are noisy, have varying degrees of sparseness and present batch effects. This high lights the need for the construction of generative models of such data that can be used for denoising and further downstream tasks. We propose a deep learning framework for unsupervised learning of cell expression models which makes use of the available spatial information to improve noise estimates. To demonstrate the novelability ofthis model to capture subcellular structure in omicsdata, we first apply the method to a simulated dataset with controlled patterns of variation. We then train models on high-resolution multi-omics spatial data and show that cell-level expression estimates are more robust when integrating spatial information, as compared to a non-spatial version of our models or state-of-the-art cell-level deep generative models.

Contributed talk

We also show that our models learn complex intercellular patterns in multi-omics data, for instance making it possible to impute expression estimates for a cell just from the interactions with its cell neighborhood space. Inconclusion, we show that ideas from popular deep generative modeling of single-cell dissociated data can be translated to the context of spatial molecular profiles, leading to more robust models and enabling the unsupervised discovery of intra- and intercellular patterns of expression.

Contributed talk

SEBASTIAAN VANUYTVEN

KU Leuven

"Single-cell genome-plus-transcriptome sequencing without upfront genome preamplification reveals differential cell state plasticity and treatment response between genetic subclones"

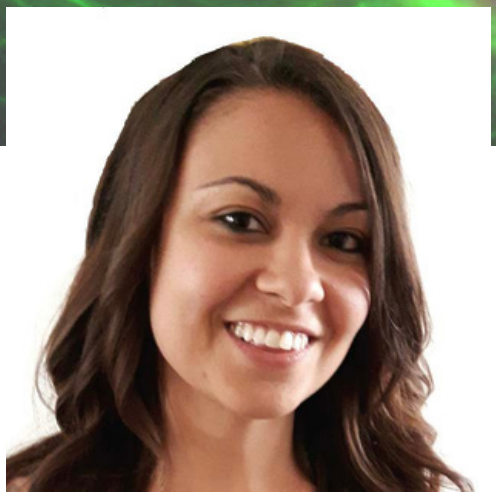
Single-cell sequencing techniques allow the study of the subclonal architecture of tumours and reveal the co-occurrence of (driver) mutations as well as their order of acquisition over molecular pseudo-time. The development of multi-omics techniques such as genome-and-transcriptome sequencing (G&T-seq) will enable us to study the diversity of cancer cell states that arises within a tumour, at its most fundamental level, the cell.

Recently we developed Gtag&T-seq, an improved G&T-seq method that applies direct genome tagmentation (Gtag), thereby avoiding upfront whole-genome amplification (WGA) and its associated cost and biases. We applied both methodologies to a human melanoma PDX melanoma model before treatment with BRAF and MEK inhibition, and at minimal residual disease. Three genomic subclones were observed at both timepoints, however their relative abundancy at cellular level changes upon drug exposure. Using the RNA of the

same single cells, we showed that transcriptome-based DNA copy number inference has limited resolution and accuracy, underlining the importance of affordable multi-omic approaches. In addition, we discerned 10 focal amplifications on chromosomes 13 and 22 of which only genes of the chr22q11.21 focal amplification showed clear gene dosage effects. Using the Gtag&T data, we were able to determine breakpoints at near base-pair resolution, which is not possible with conventional G&T-seq. Finally, we mapped known melanoma resistant cell states on top of the phylogenetic cell lineage tree revealing that some cell states do not occur in all of the subclones.

In conclusion, Gtag&T-seq is a novel, low-cost, and accurate single-cell multi-omics method that enables the exploration of somatic genetic alterations and their functional consequences in single cells at scale.

Special workshop



NOVELLA GUIDI

Life Science Alliance (EMBO Press, Rockefeller University Press, Cold Spring Harbor Laboratory Press)

"Open Science & Publishing Tips"

Writing research papers for scientific journals is not trivial and is also very competitive. This workshop will give you an overview of the open science editorial features at Life Science Alliance that foster data transparency and fast publication. Then, dive into key publishing tips on how to effectively write a good and attractive title & abstract, prepare a cover letter, successfully appeal a decision, and respond to reviewers' comments. Ultimately, few important tips will be covered on how to fairly peer review if you are the referee. Thus, this workshop offers a clear opportunity to understand what editors like and what they do not and therefore increase your chances to successfully publishing.

Sponsored talk

THOMAS AYERS

Dolomite Bio | Izasa Scientific

"Democratizing single cell research"

Single cell research is here to stay, with new advances in single cell sequencing being developed every day. A new challenge is not just to make single cell workflows possible, but to make them accessible to the greatest number of people.

A major barrier to the accessibility of single cell techniques is cost, as with many genomic technologies. Single cell workflows are inherently expensive, as products of multiple high-precision instruments from dissociation to bioinformatic analysis are strung together in sequence. The fair and widespread adoption of single cell RNA-sequencing in clinical, basic, and industrial research settings is heavily dependent on these costs being brought down.

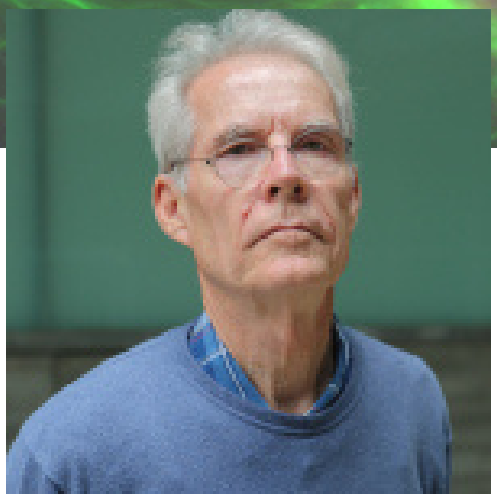
As the field of Single Cell matures, Dolomite Bio recognises a need to make solutions affordable to all labs, not just those that can afford to dedicate budget towards expensive commercial kits.

Sponsored talk

Accessibility doesn’t just take the form of cost, but also sample compatibility with more than just standard cell types. Differences in cell morphology require robust technology to assay all cell types in an unbiased way. This is especially important in single cell work, where maintaining the heterogeneity of a tissue is crucial to research questions.

The widespread adoption of single cell workflows is also tied to the open-source movement within science, as more researchers look to using their own user-sourced reagents, sharing tweaks and protocol optimisations as they go.

Here we present a robust kit and platform solution in the form of the Nadia instrument and RNAdia kit which enables cost reduction, convenience, reagent flexibility and wide sample compatibility compared to other existing single cell RNA-Sequencing solutions.

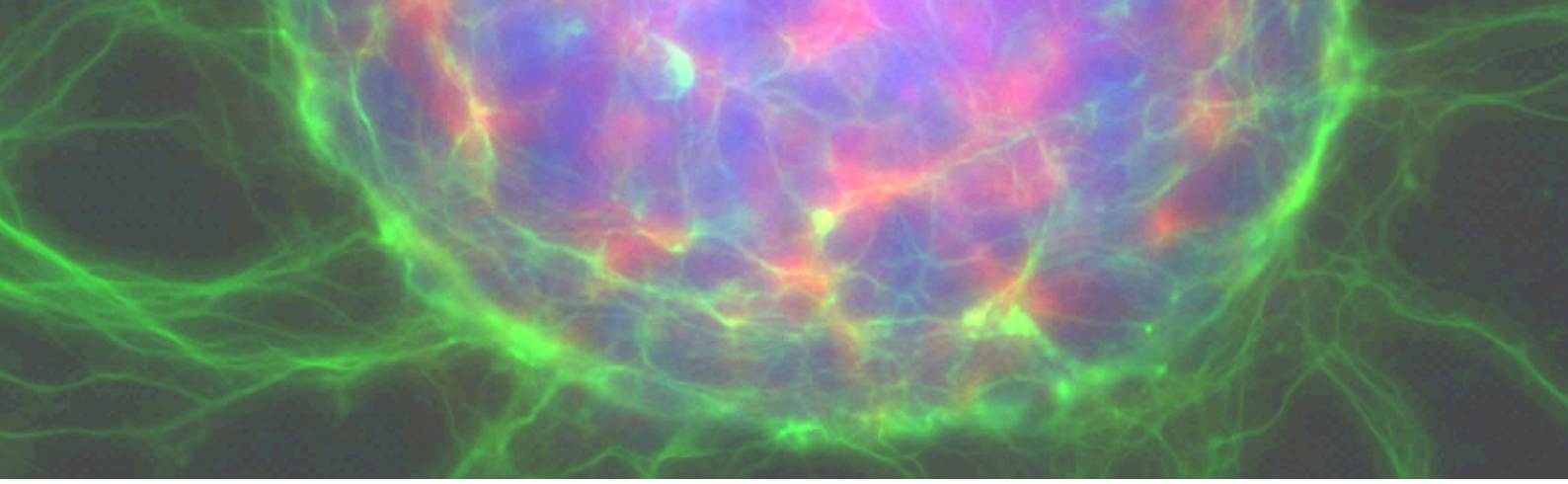


THOMAS GRAF

Centre for Genomic Regulation (CRG)

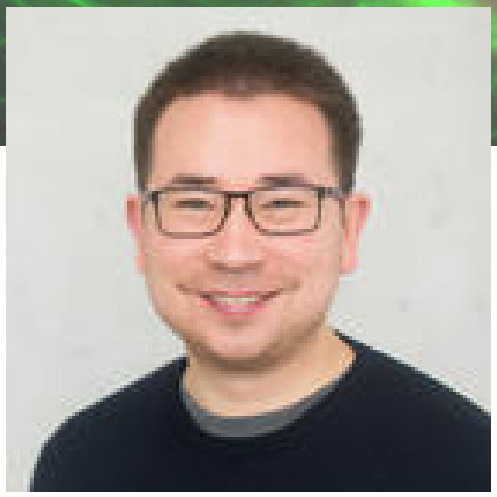
"Transcriptional regulation of the trophectoderm-ICM bifurcation during pre-implantation embryo development"

The earliest cell fate decision during development occurs in pre-implantation embryos, when totipotent cells differentiate into either the inner cell mass (ICM) cells, becoming the embryo proper or the trophectoderm (TE), destined to become the placenta. Although a number of transcription factors (TFs) are known to specify TE and ICM cells, what drives the earliest asymmetry is poorly understood. We have now identified C/EBPa as strong candidate that plays such a role, based on our finding that the factor powerfully enhances the reprogramming of B cells into both iPS cells and TE-like cells after subsequent induction of the Yamanaka factors (OSKM). We also discovered that C/EBPa is asymmetrically expressed in the vegetal pole blastomere of 4 cell embryos, known to be biased towards TE differentiation as well as in the trophectoderm layer of blastocysts. In line with a function as a novel specifier of TE cell fate, the ectopic expression of C/EBPa in embryonic stem cells (ESCs) induces their differentiation into TE-like cells.



Single cell analyses revealed two major branches of induced developmental trajectories: one towards TE- like cells and another one to cells that exhibit characteristics of the ICM. Concomitantly, we observed the disappearance of 2C like cells, known to be present in ESC cultures, and the presence of a small cluster of cells expressing primitive endoderm genes. Our data indicate that C/EBPa is a driver of the earliest developmental decision and a so far unappreciated regulator of trophectoderm fate.

Contributed talk

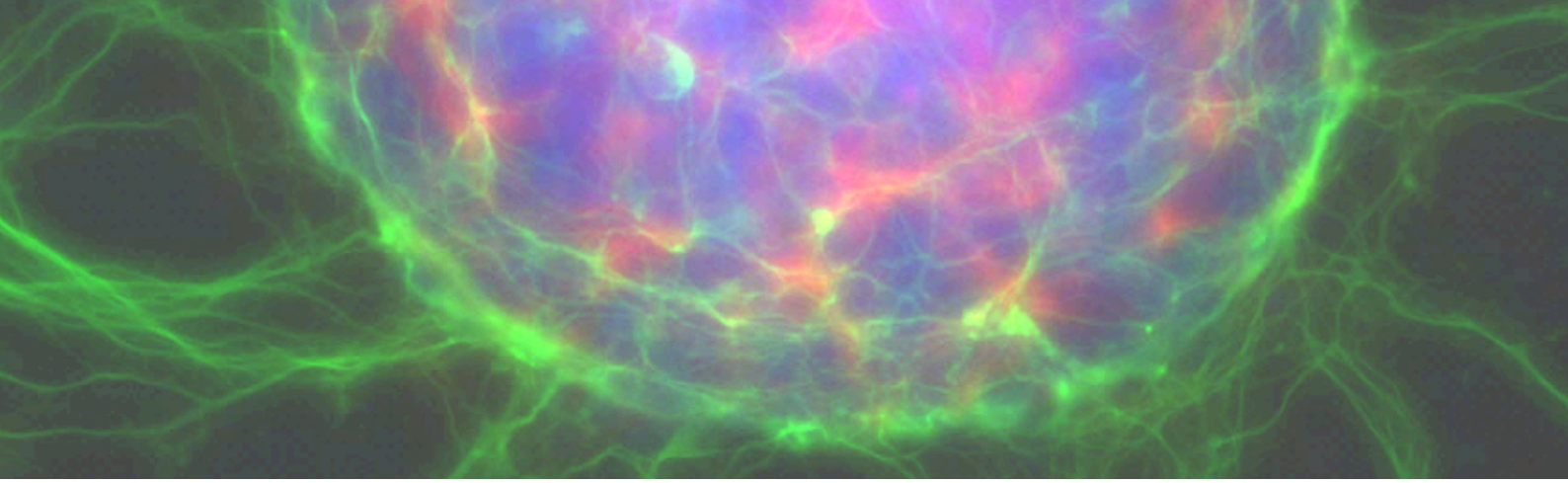


LEIF LUDWIG

Berlin Institute of Health

"Single-cell multi-omics reveal routes of purifying selection and metabolic vulnerabilities due to pathogenic mitochondrial DNA"

Alterations in mitochondrial DNA (mtDNA)are associated with a spectrum of often multi-systemic disorders, but also cell-type specific defects. Large deletions in mtDNA have been linked to a variety of clinical pathologies, including newly arisingin congenital disorders such as Pearson syndrome(MIM:557000), a mitochondrial disease characterized by sideroblastic anemia and exocrine pancreas dysfunction. Here, we establish a multi-omics approach to quantify mtDNA deletion heteroplasmy and features of cell state in thousands of single cells. By profiling primary hematopoietic cells from three patients with Pearson syndrome, we observe widespread transcriptional changes and altered AP-1 transcription factor activity. We resolve the interdependence between pathogenic mtDNA heteroplasmy and cell lineage, including purifying selection against mtDNA deletions in effector-memory CD8 T cell populations,further suggesting cell state specific metabolic vulnerabilities.



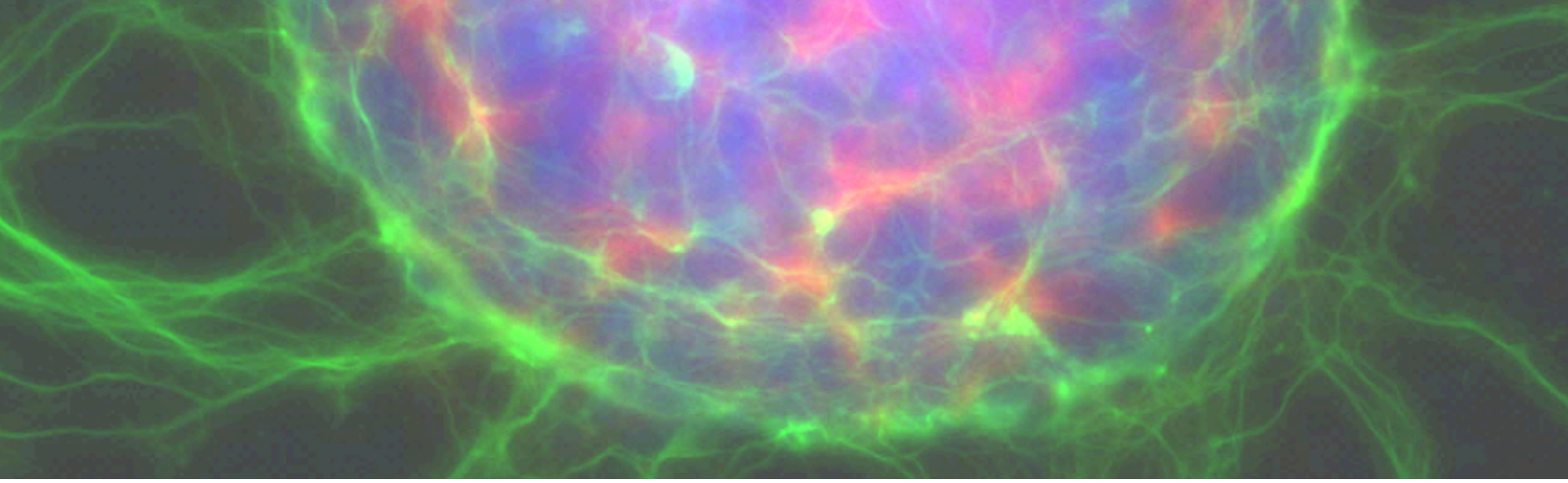
Additionally, single-cell analyses of in vivoand in vitrodifferentiated bone marrow mononuclear cells reveal multi-faceted clonal dynamics in a patient with both Pearson syndrome and del7q myelodysplastic syndrome (MDS). Our results identify genomic perturbations underlying this mitochondrial disorder and more generally provide a multi-omicsframework to the study of human disease evolution at single-cell resolution.

ALEJO RODRIGUEZ-FRATICELLI

Institute for Research in Biomedicine (IRB Barcelona)

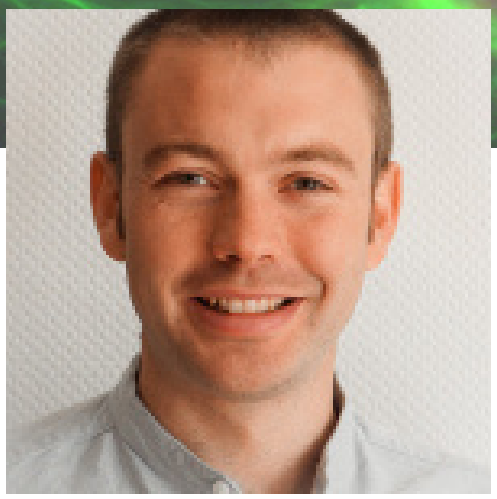
"Single-cell lineage analysis of aged and premalignant hematopoietic stem cells"

For decades, biologists have noted that stem cells in adult tissues show extensive functional heterogeneity. However, these functional behaviors are strikingly similar when comparing hematopoietic stem cells that share a common ancestor, suggesting that this heterogeneity is maintained by intrinsic and heritable properties. Despite these observations, a detailed understanding of the mechanisms driving the variation in tissue stem cell behaviors has remained elusive. Others and we have previously shown that expressed barcodes can be used to simultaneously obtain the cellstate and lineage ancestry of thousands of single cells at a time. Using these technologies we have carried out dynamic analysis of states and fates for thousands of differentiating clones in parallel and revealed novel regulators of fate decisions. Interestingly, serial stem cell transplantation experiments revealed intrinsic and heritable states driving differences in the balance between stem cell self-renewal and differentiation. Alterations of this balance is central to two conditions



where hematopoietic stem cells are known to play a major role: hematopoietic aging and malignant disease.

Using single-cell lineage tracing, we find that both aged and preleukemic HSCs are characterized by small subsets of clones that expand and self-renew massively with reduced differentiation capacity. Intriguingly, age-related and mutation-driven clonal states appear to drive selective self-renewal advantages through different mechanisms. In sum, we show that connecting cellular states and cellular fates through high-resolution lineage tracing can be used to define drivers for a variety of therapeutically relevant stem cell properties



LARS VELTEN

Centre for Genomic Regulation (CRG)

"Clonally resolved single-cell multi-omics enables the identification of cancer specific surface antigens"

Single cell RNA-seq is a powerful method for the characterization of intra-tumor heterogeneity, however, it usually lacks information on clonal identities and does not enable clean, intra-sample comparisons between cancerous and cell-type matched healthy cells. Here, we introduce MutaSeq v2, a protocol for clonally resolved multi-omics that can be applied to already existing cDNA libraries from the 10x genomics platform. MutaSeq v2 amplifies mitochondrial and selected nuclear SNPs, infers copy number variation, and ultimately estimates clonal hierarchies and single-cell clonal status through a Bayesian model that accounts for the noise properties of single-cell RNA-seq data. We demonstrate MutaSeq v2 in the context of acute myeloid leukemia (AML), where the similarity between healthy and leukemic cells poses a diagnostic challenge. Our data identifies novel differentiation state-independent surface markers for leukemic and healthy cells, respectively, suggesting a novel, rapid strategy for the estimation of leukemia content of bone marrow samples. Together, our data illustrate the power of clonally resolved single cell multi-omics.

ADAM MEAD

University of Oxford

Deciphering TP53 mutant Cancer Evolution with Single-Cell Multi-Omics

TP53 is the most commonly mutated gene in human cancer, typically occurring in association with complex cytogenetics and dismal outcomes. Understanding the genetic and non-genetic determinants of TP53-mutation driven clonal evolution and subsequent transformation is a crucial step towards the design of rational therapeutic strategies. Here, we carry out allelic resolution single-cell multi-omic analysis of haematopoietic stem/progenitor cells (HSPC) from patients with a myeloproliferative neoplasm who transform to TP53-mutant secondary acute myeloid leukaemia (AML), a tractable model of TP53-mutant cancer evolution. All patients showed dominant TP53 ‘multi-hit’ HSPC clones at transformation, with a leukaemia stem cell transcriptional signature strongly predictive of adverse outcome in independent cohorts, across both TP53-mutant and wild-type AML. Through analysis of serial samples and antecedent TP53-heterozygous clones, we demonstrate a hitherto unrecognised effect of chronic inflammation, which supressed TP53 wild-type HSPC whilst enhancing the fitness advantage of TP53 mutant cells. Our findings will facilitate the development of risk-stratification, early detection and treatment strategies for TP53-mutant leukaemia, and are of broader relevance to other cancer types.

Contributed talk

DIRENA ALONSO

IRB Barcelona

"Heterotypic cell interactions direct neoplastic competence within the pre-malignant pancreas"

The oncogenic potential of cancer mutations and inflammation can vary greatly even amongst morphologically indistinguishable cells, which may appear normal yet harbor oncogenic drivers such as mutant KRAS. To define molecular and cellulardeterminants of neoplastic competence at high resolution, we used single-cell sequencing to comprehensively profile normal, inflamed, pre-cancerous and malignant epithelia inautochthonous mouse models of pancreatic allowingselective tracing, enrichment and perturbation of epithelial cells exposed to pro-oncogenic genetic and inflammatory insults. Integrative analyses of transcriptomic and epigenomic single-cell data revealednon-stochastic epigenetic heterogeneity within the pre-malignant epithelium, and uncovereddiscretesubpopulations of inflammation-sensitive KRAS-mutant cells that are primed by non-linear chromatin reprogrammingeventsfor diverse benign and malignant lineages emerging during tumor evolution. We find that greaterepigeneticpotentialfor precursorstates and full-blown pancreatic canceris associated with

Contributed talk

specific cell-cell communication programs. These programs become epigenetically-activated by oncogenic KRAS and inflammation in a lineage-specific manner,andalso define phenotypic diversity in advanced human cancers.To identifycell-cell communication networksdirectingearly progression, we developed a novel algorithm for gene-module-based inference of cell-cell crosstalkand performed spatiotemporally-controlled genetic perturbations in the pre-malignant epitheliacoupled with single-cell sequencing. As predicted by our computationalanalyses, perturbationofnetworksinterconnecting inflammation-sensitive KRAS-mutant epithelial cells with their immune environment impairs the emergence of tumorigenesis-associated lineages.Our results causally implicate epigenetic plasticity in the establishment of tissue-level cell-cell interactions that define and directneoplastic competence, and chart non-linear epigenetic roadmaps to pancreatic cancer and its precursors.

Contributed talk

SERGIO MARCO-SALAS

Stockholm University

"Developmental origins of cell heterogeneity in the human lung"

The lung contains numerous specialized cell-types with distinct roles in tissue function and integrity. To clarify the origins and mechanisms generating cell heterogeneity, we created a first comprehensive topographic atlas of early human lung development.We integrated scRNA-Seq and different spatially-resolvedtranscriptomicstechnologies such as In Situ Sequencing, Spatial transcriptomicsand SCRINSHOTinto a web-based, open platform for interactive exploration. We report 83 cell states, several spatially-resolved developmental trajectories and predict cell interactions within defined tissue niches. Toillustrate the utility of our approach we show distinct states of secretory and neuroendocrine cells, largely overlapping with the programs activated either during lung fibrosis or small cell lung cancer progression. We define the origin of uncharacterized airway fibroblasts associated with airway smooth muscle in bronchovascular bundles, and describe a trajectory of Schwann cell progenitors to intrinsic parasympathetic neurons controlling bronchoconstriction. Our atlas provides a rich resource for furtherresearch and a reference for defining deviations from homeostatic and repair mechanisms leading to pulmonary diseases.

OLIVER STEGLE

German Cancer Reseach Center

"From genotype to phenotype with single-cell resolution"

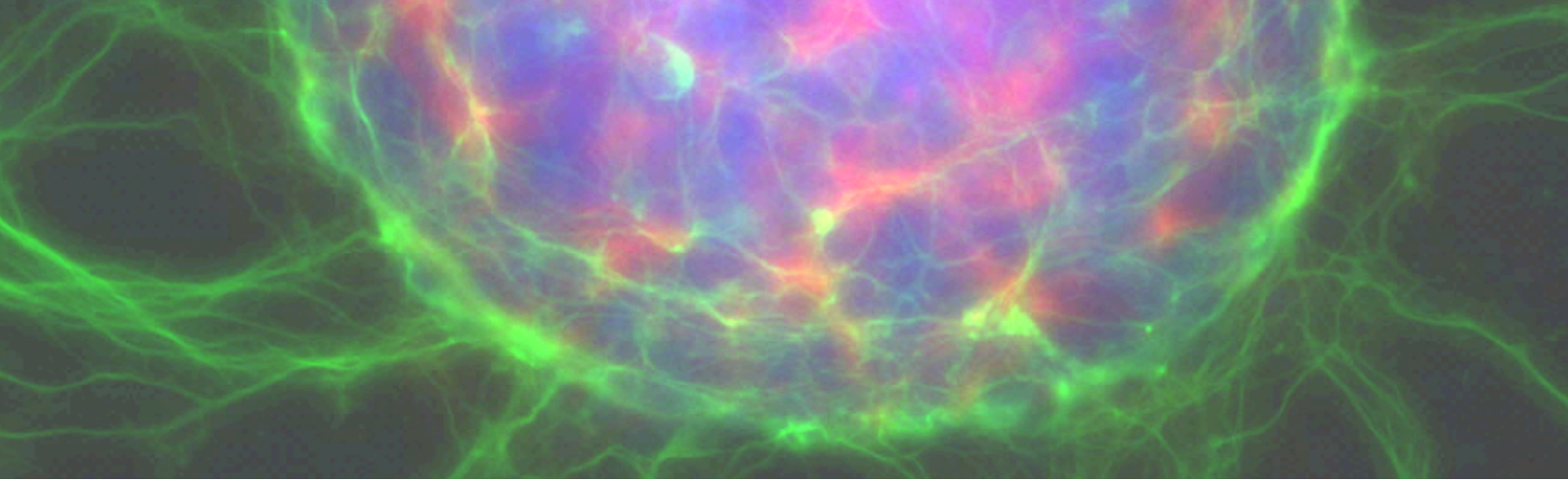
The study of genetic effects on gene expression and other molecular traits using bulk sequencing has allowed for the functional annotation of disease variants in diverse human tissues. Advances in single-cell RNA sequencing and multi-omics protocols provide for unprecedented opportunities to greatly increase the resolution of such genetic analyses, allowing to assess gene regulatory effects at the resolution of cell types, cell states and even in individual cells in human tissues. In this talk, I will present computational strategies for analyzing and integrating population-scale multi-omics dataset. I will then describe applications of these strategies to population-scale single-cell sequencing dataset from genetically diverse human iPSCs across differentiation towards a neuronal fate. Our data provide unprecedented opportunities to map regulatory variants and human disease variants both in major cell types but also in subtle subtypes and across cellular differentiation, revealing dynamic changes of regulatory variants. We describe novel disease-relevant linkages of several of the regulatory variants identified, thereby illustrating how this technology can open novel opportunities to study risk factors for human diseases.

ANAMARIA ELEK

Centre for Genomic Regulation (CRG)

"Single cell approaches for compartive and regulatory characterization of major animal cell types"

Cell types are basic functional and evolutionary units of animal multicellularity. Single-cell RNA-seq methods have enabled generation of cell atlases for many model species, with transcriptomic profiles of their various tissues and organs now mapped out in great detail. In early-branching animal lineages - which include sponges, ctenophores, placozoans and cnidarians - single-cell methods can be applied to whole organisms, owing to their relatively small body size and smaller number of major cell types. This enables unbiased characterization of their cell repertoires, and paves the way for comparative analyses that can shed light on the evolution of major animal cell types. We used scRNA-seq to identify similarities and divergences of cellular repertoires within and between species of cnidarians that diverged from a common ancestor 500 millions of years ago, and we show that, while broad cell types identities are strikingly well conserved between species, the individual cell types defined within these broad identities have undergone a fast evolutionary diversification.



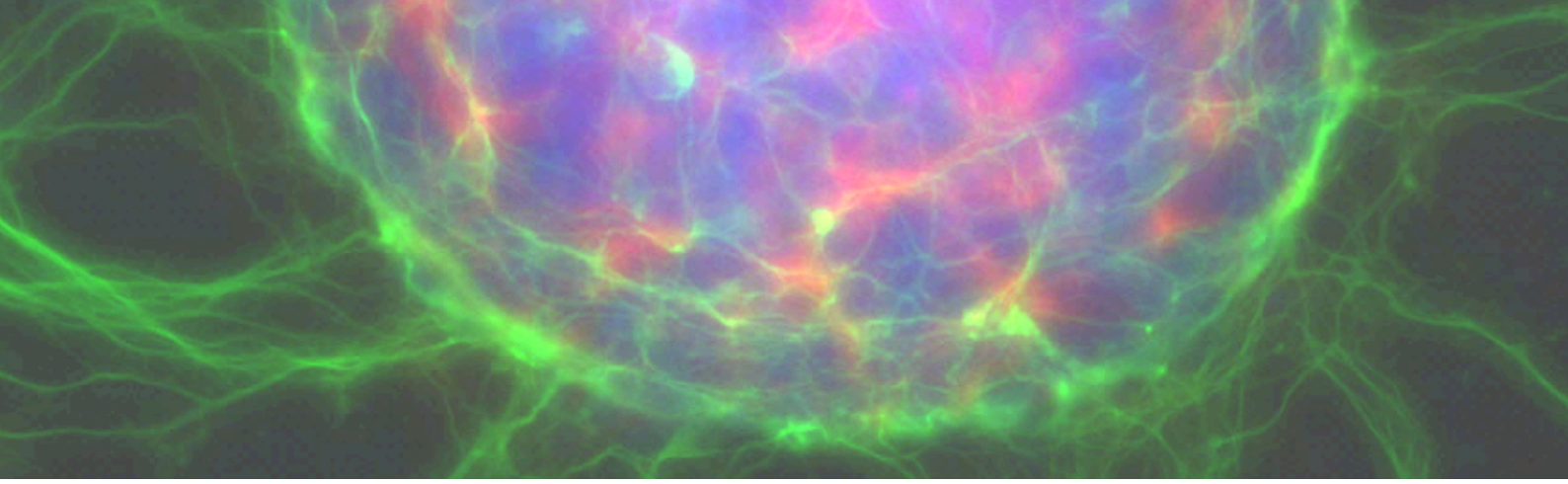
Next, by integrating gene expression and chromatin accessibility, we aim to understand gene regulatory logic driving the individuation of particular cell types. While most transcription factors (TFs) are known to be active across many cell types, we are focusing on identifying combinations of TFs and their target genes - organized in gene regulatory modules or networks - and quantifying the ways in which they are specifically deployed in a particular cell type to define its transcriptional identity.

MARIONA NADA-RIBELLES

IRB Barcelona

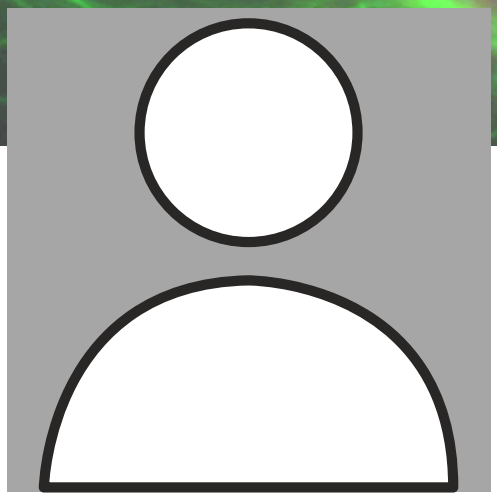
"Untangling the structure of gene regulatory networks during adaptation"

How single cell variability reflects onto the phenotypic spectrum remains largely unknown. Even in clonal yeast populations, gene expression variability is key to explaining divergent cell fates to extracellular perturbations. Adaptation to environmental insults is driven by the integration of signals by stress activated protein kinases (SAPKs). In response to osmotic stress, the activation of the Hog1/p38 SAPK pathway overrides all cellular processes including the rewiring of gene regulatory networks to promote survival, which pushes cells from a relatively stable (adapted) state to a transient unstable state until cellular homeostasis is restored (adaptation). Stress-induced transcription is nosier than steady-state conditions; therefore, this non-genetic heterogeneity provides cells the ability to produce discretely distinct phenotypes during adaptation. Here we have combined traceable genetic perturbations of known regulators of the osmoadaptive response in yeast with single cell transcriptomics to define the transcriptional paths to stress adaptation.



By dissecting the contribution of each individual cell, we could observe distinct usages of the canonical osmostress response accompanied by distinct transcriptional signatures within genotypes. Our findings expose the layers of transcriptome complexity during adaptation and the degree of diversification of transcription programs.

Sponsored talk



CHRISTOPHE FLEURY

10xGenomics | Bonsai Lab

"New Perspectives in Single Cell: Pushing the Boundaries with 10x Genomics Solutions"

Single cell sequencing technologies have transformed our understanding of biology by letting us profile tissue heterogeneity with powerful resolution. To reach new insights into complex biology, researchers need next generation multiomics — the ability to capture multiple measurements simultaneously from the same single cell.
Hear about the latest breakthroughs and innovations in single cell multiomic profiling of combined transcriptome, epigenome or protein and how these methods have uncovered novel insights in cancer research, neuroscience and more.

Learn how Chromium Single Cell Solutions from 10x Genomics can help you push the boundaries of your research. Resolve highly complex biological systems at true resolution.

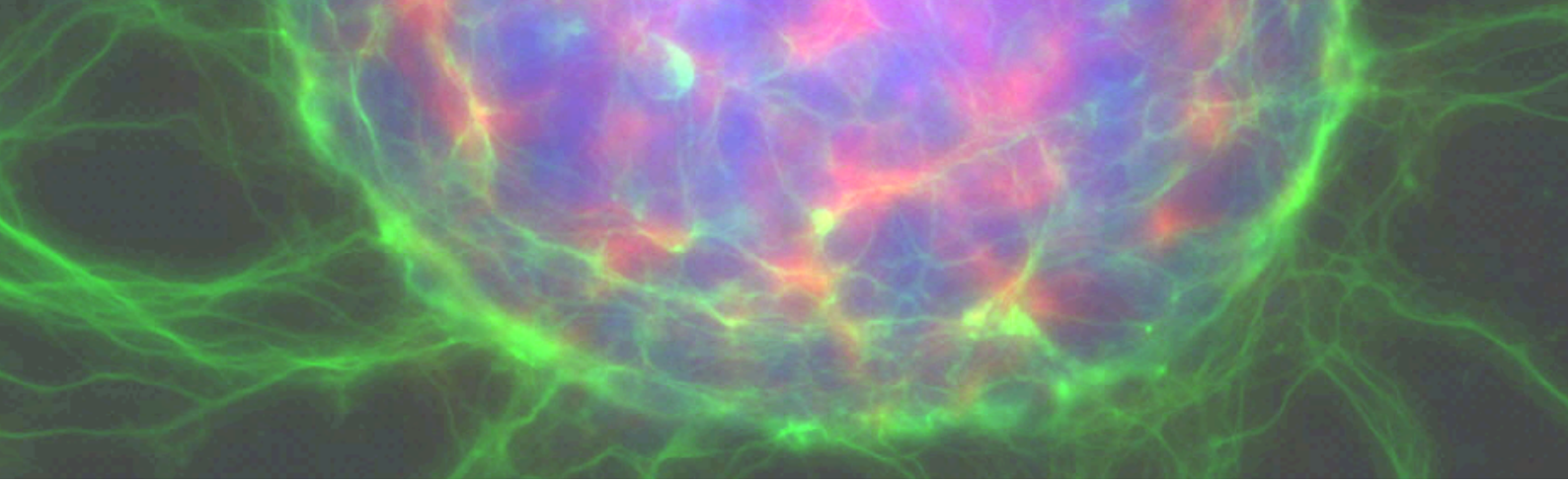
MIREYA PLASS

Bellvitge Biomedical Research Institute (IDIBELL)

"Single-cell transcriptomics of iPSC-derived neurons reveals functional changes in Alzheimer's Disease"

Alzheimer’s disease (AD) is the most common age-related neurodegenerative disease that heavily burdens healthcare systems worldwide. Most AD patients are sporadic and despite all the efforts, we still do not know the molecular mechanisms triggering the development of AD. One of the main problems to study AD and other neurodegenerative diseases is the lack of good experimental models that recapitulate the pathological features of the disease. In that context, induced pluripotent stem cell (iPSC) technology has provided an excellent tool to model disease pathogenesis considering the patients’ genetic background.

In this project, we have used single-cell transcriptomics to study the molecular changes that happen during the differentiation of iPSCs derived from sporadic AD patients to neurons. Preliminary results show that AD-derived neural progenitor cells already show changes in the expression of genes previously associated with AD or related to neuronal differentiation and RNA processing.



These results demonstrate that neurons from sporadic AD patients show transcriptomic differences before the onset of the disease and thus can be used as a relevant model to study the molecular networks driving AD. Future work will be directed to validate these findings and assess its impact in the development of AD.

Taken together, our results that the combination of iPSC technology and scRNA-seq is a potent tool for the study of the molecular mechanisms triggering the development of neurodegenerative diseases such as AD.

MUZZ HANIFFA

Newcastle University & Wellcome Sanger Institute

"Decoding the developing human immune system"

Muzlifah has used functional genomics, comparative biology and single cell RNA sequencing to study human mononuclear phagocytes. In this seminar, she will demonstrate the applications of single cell genomics to decode the developing human immune system.

Poster session

MIROSLAVA K. ADAMCOVA

Institute of Molecular Genetics of the Czech Academy of Sciences

Emergency granulopoiesis induces a lymphoid to myeloid bias switch in a subset of hematopoietic stem cells.

Granulocytes represent the first line of defense against bacteria and funghi, thus their production needs to be adapted to specific demands. While daily production of granulocytes is sustained by steady-state granulopoiesis, upon infection this program switches to emergency granulopoiesis (EG), to ensure enhanced and accelerated granulocytic production. Recently, it was shown that unperturbed hematopoiesis is sustained by multipotent progenitors, however, during stress conditions, hematopoietic stem cells (HSCs) become active and responsible for coping with stress situations. Yet, whether and how HSCs play a role during EG is unknown. To understand the contribution of HSCs to the EG response, we performed single cell RNA sequencing analysis of sorted murine HSCs (Lin-c-Kit+Sca-1+CD48-CD150+) 4 hours after lipopolysaccharide (LPS) stimulation, which mimics a bacterial infection and activates EG in vivo. Strikingly, we observed radical changes in the HSC cluster composition between PBS control and LPS treated mice. Interestingly, these changing population dynamics were marked by alterations in HSC lineage bias, demonstrating that under EG there is an expansion of the myeloid-bias HSCs at expenses of the lymphoid-bias

Poster session

HSCs. Remarkably, we identified Procr (CD201) as a specific cell surface marker for this lymphoid to myeloid transition, allowing us to distinguish, sort and track the switch from CD201+ lymphoid-bias HSCs (present in PBS control) towards CD201- myeloid-bias HSCs (present upon LPS stimulation). Further, the abundant CD201- myeloid-bias HSC clusters were characterized by an inflammatory gene signature. Particularly interesting, was the elevated expression of Wnt10b, which we verified by qRT-PCR, ELISA, and microscopy. We demonstrated that Wnt10b activates the canonical Wnt/ -catenin signaling pathway and promotes EG. In conclusion, we observed that HSCs actively take part in early stages of EG by activating an inflammatory signature associated with the switch from lymphoid to myeloid bias, therefore ensuring sufficient production of myeloid cells to fight the infection.

Poster session

FRANZ AKE

bellvitge Institute for BiomedicalResearch (IDIBELL)

Characterisation of alternative polyadenylation at single cell resolution in Alzeheimer disease

Alternative polyadenylation (APA) is a widespread mechanism of gene regulation that generates mRNA isoforms with distinct 3'ends. APA is well known to be regulated during cell differentiation and is a major source of gene regulation in the brain. Proliferating cells tend to have shorter 3'UTRs while differentiated cells have longer 3'UTRs. Changes in APA patterns are not only characteristic of cellular differentiation but also have been associated with pathological processes such as cancer or neurodegenerative diseases like Alzheimer's disease(AD). Therapid development of 3'tag-basedsingle-cell RNA sequencing (scRNAseq) has enabled the study of gene expression at the individual cell level and the implementation of methods for describing APA site sat single cell resolution. Here we present PLAPA, 0 a tool for characterising APA site sat single cell resolution using 10XGenomics or Dropseqsc RNA-seqdataset. PLAPA allows quantifying RNA expression at isoform levela nds ingle-cell resolution and identifying changes in isoform usage across cell populations and conditions. We used PLAPA to study the changes in APA during the differentiation of induced pluripotent stem cells (iPSCs) to neuroprogenitor cells(NPCs). The results from our analysis show clear changes in 3'end usage between iPSCs and NPCs. We project to use PLAPA to investigate the role of APA in neural differentiation and itsr ole in the development of AD and how APA changes during neural differentiation and how these changes are altered in AD.

Poster session

AYBUGE ALTAY

Annotating scATAC-seq pseudobulk clusters

Chromatin structure can control the accessibility of potential gene regulatory elements in a dynamic and cell type-specific manner and therefore plays a critical role in gene regulation. Although accessibility can be measured by “bulk” technologies like ATAC-seq providing a whole genome readout, these technologies cannot characterize the unique properties of single cells. Recent advances in the field have enabled methods in single-cell (sc) resolution, however, these methods suffer from the sparsity of the resulting data. Annotating cell types in scATAC-seq data remains a challenge mainly due to the lack of marker open regions to characterize cell types. In this study, we create scATAC-seq pseudobulk clusters by summing up the reads in a cluster. We then use bulk ATAC-seq to annotate these “pseudobulks” using the same embedding space. This strategy provides a feasible way to overcome sparsity and leverages a large number of characterized ATAC-seq data. Here we applied our approach to human primary blood and brain data and coupled the analysis with TF-footprinting. Our pipeline noticeably resolves the cell-type annotation problem demonstrating the strength of our strategy.

Poster session

RICARD ARGELAGUET

Babraham Institute

A multi-omics roadmap of mouse early organogenesis

During gastrulation a single-layered blastula is reorganised to give rise to the three primary germ layers, which in turn will develop into the major organs in a process known as organogenesis. Single-cell sequencing technologies have enabled the profilingof molecular programmes during embryonic development in a variety of model organisms. Nevertheless, without multi-modal measurements from the same cell it is difficult to unambiguously infer the interplay between gene expression, transcriptional regulators and cis-regulatory DNA sequences.Here we generate a multi-omics atlas of mouse early organogenesis by simultaneously profiling gene expression and chromatin accessibility from tens of thousands of cells. We provide a molecular characterisation of lineage-specific regulatory programmes. In addition we exploit the multi-modal readouts to quantify transcription factor activities and to infer gene regulatory networks that underpin cell fate decisions.

Poster session

DIEGO BALBOA

University of Helsinki

Functional, metabolic and transcriptional maturation of stem cell derived beta cells

Transplantation of pancreatic islet cells derived from human pluripotent stem cells isapromising treatmentfor diabetes. Despite progress in generation of stem cell-derived islets(SC-islets), detailedcharacterization of their functional propertieshas not been conducted. Here, we generated functionally matureSC-islets using an optimized protocol and comprehensively benchmarked them against primaryadultislets.Biphasic glucose stimulated insulin secretion developed during in vitromaturation, associated with cytoarchitectural reorganizationand increased alpha cells. Electrophysiology, signaling and exocytosis of SC-islets werecomparable to adult islets. Glucose-responsive insulin secretion was achieved despite differences in glycolytic and mitochondrial glucose metabolism. To investigatethe transcriptionalchangesassociated within vitroand in vivoSC-islet maturation,we performed single cell RNA sequencing onfour timepoints of SC-islet in vitrodifferentiation as well as SC-islet grafts retrieved at 1-, 3-and 6 months post-engraftment, and compared them with primary adult islets. These single cell transcriptomic analysesrevealed a continuous maturation trajectory culminating in a transcriptional landscape closely resembling that of primary islets. In summary, ourresultsindicatethat a remarkably high level of betacell functionality is achieved in vitro, even if specific metabolic and transcriptomic differences persist between SC-islet beta cells and primary beta cells. Thisthorough evaluationof SC-islet maturation highlights their advanced degree of functionality and supports their use in further efforts to understand and combat diabetes.

Poster session

AGOSTINA BIANCHI

Centre for Genomic Regulation - Universitat Pompeu Fabra

Single-cell high-throughput targeted DNA methylation analysis for uncovering epigenetic heterogeneity

DNA methylation at cytosine-guanine dinucleotides (CpGs) is a cell-type-specific epigenetic mark that plays a critical role during development. It has motivated the advancement of single-cell DNA methylation analysis methods to assess cellular identity as well as epigenetic heterogeneity. However, due to the extensive sequencing effort required to cover the large number of CpGs in the human genome, currently available techniques produce extremely sparse datasets with low cellular throughput. To tackle this, we developed a high-throughput targeted method, which facilitates the assessment of up to 600 CpGs, in several thousand single cells (single-cell Targeted Analysis of the Methylome, scTAM-seq). This novel approach combines PCR amplification in droplets (Tapestri platform from Mission Bio) with digestions by a methylation-sensitive restriction endonuclease.

We applied scTAM-seq to distinguish the cellular identity of a mixture of two malignant hematopoietic cell lines and to study the DNA methylation profile of B-cell-specific CpGs in B-cell subpopulations from peripheral blood. scTAM-seq identified cell types based on their DNA methylation profile at unprecedented resolution. Cluster's pseudobulk DNA methylation values obtained with scTAM-seq strongly correlated with bulk DNA methylation data ($R2 > 0.85$). Moreover, by focusing on about 600 CpGs of interest, scTAM-seq yielded DNA methylation values of every locus in every cell, with a moderate incidence of dropout, while reducing the sequencing requirements. Due to the design of the Mission Bio Tapestri platform, we will also be able to combine DNA methylation readouts with mutational data and cell surface markers expression, deciphering multiple layers of information from the same cell.

Poster session

ADRIÀ CABALLÉ

Technical and biological influence of ribosomal RNA on single cell RNA data analysis

Ribosomal RNA (rRNA) accounts for 80–90% of the total RNA content in typical mammalian cells. Despite most existing scRNA-seq methods only capture polyadenylated mRNA, ribosomal reads can still represent a large fraction of the total library size, reaching up to 50% in some experiments. Importantly, the percentage of ribosomal reads varies widely not only across datasets but also across individual cells and cell populations within a given experiment. This cell-to-cell variability has an influence on the gene expression versus sequencing depth relationship, which is at the basis of widely used normalization and variance stabilization methods such as Seurat's sctransform. For several independent datasets, we compared the goodness of fit of the underlying model in sctransform applied to the full data and also when ignoring ribosomal genes. In most of the cases, removing rRNA reduced the variance of the Pearson residuals of regularized negative binomial regression, thereby explaining more of the data's variance. However, variability in ribosomal gene expression can sometimes be biologically relevant, therefore we developed an alternative approach to reduce the technical influence of ribosomal content on normalization while preserving ribosomal genes for downstream analyses. To address whether our procedure prevents from detecting biological signal associated with ribosomal activity, we compared the results obtained before and after rRNA removal. We found that we could still distinguish between cell populations representing different biosynthetic states. Altogether, we raise awareness about the influence of ribosomal content in scRNA-seq data analysis and provide informative metrics and plots for assessment of new datasets.

ADRIÀ CABALLÉ

Centre for Genomic Regulation - Universitat Pompeu Fabra

Technical and biological influence of ribosomal RNA on single cell RNA data analysis

Ribosomal RNA (rRNA) accounts for 80–90% of the total RNA content in typical mammalian cells. Despite most existing scRNA-seq methods only capture polyadenylated mRNA, ribosomal reads can still represent a large fraction of the total library size, reaching up to 50% in some experiments. Importantly, the percentage of ribosomal reads varies widely not only across datasets but also across individual cells and cell populations within a given experiment. This cell-to-cell variability has an influence on the gene expression versus sequencing depth relationship, which is at the basis of widely used normalization and variance stabilization methods such as Seurat's sctransform. For several independent datasets, we compared the goodness of fit of the underlying model in sctransform applied to the full data and also when ignoring ribosomal genes. In most of the cases, removing rRNA reduced the variance of the Pearson residuals of regularized negative binomial regression, thereby explaining more of the data's variance. However, variability in ribosomal gene expression can sometimes be biologically relevant, therefore we developed an alternative approach to reduce the technical influence of ribosomal content on normalization while preserving ribosomal genes for downstream analyses. To address whether our procedure prevents from detecting biological signal associated with ribosomal activity, we compared the results obtained before and after rRNA removal. We found that we could still distinguish between cell populations representing different biosynthetic states. Altogether, we raise awareness about the influence of ribosomal content in scRNA-seq data analysis and provide informative metrics and plots for assessment of new datasets.

Poster session

VIKRAM DEVGAN

NanoString Technologies

"Subcellular expression map of ~1000 genes and spatially-resolved cell type map of non-small cell lung cancer tissues using CosMx Spatial Molecular Imager "

Understanding the multi-cellular spatial interactions that create organs and organisms (in both health and disease) is one of the key challenges of modern biology. Advanced sequencing technologies have allowed the analysis of large numbers of dissociated cells, enabling projects to analyze all the cell types in individual organs. However, dissociated cells lose the spatial information on cellular organization and interaction. The spatial arrangement of cells and their local spatial environments is increasingly recognized as being essential to understanding biological function in both health and disease. The non-small cell lung cancer (NSCLC) atlas will measure more than 1000 different RNA targets simultaneously in-situ, with subcellular resolution and spatial mapping in 3 dimensions. The CosMx SMI system was used to analyze FFPE samples from 5 NSCLC patients, generating data on over 800,000 cells. To allow the entire scientific community to explore these data, and develop new analysis methodologies and algorithms, the data has been made available to all researchers. To our knowledge, this is the l

Poster session

the largest biobanked sample high-plex RNA data set ever collected. This data is now available to all researchers to allow the entire scientific community to develop new analysis methodologies and discover new insights into NSCLC.

Our initial analysis of this data reveals tumor biology in unprecedented sub-cellular and single-cell spatial detail. Using this dataset, we have observed the complex ways in which 18 immune cell populations invade tumors: in one tumor, memory CD8 T-cells invade broadly throughout the tumor, while neutrophils gather densely in large stroma areas. The data enable the delineation of niches within the tumor microenvironment, such as lymphoid structures packed with B-cells and T-cells, pockets of dense macrophages, stroma dominated by two subpopulations of fibroblasts, and tumor interior with only sparsely invading immune cells. We describe how immune cells change behavior across spatial contexts, contrasting expression of macrophages invading diffusely into the tumor and macrophages gathering densely in the stroma. Furthermore, we characterize the interaction of tumor cells and the environment, e.g. one tumor up-regulates VEGFA and NRDG1 only when contacting stroma.

Poster session

JOHANNA GEUDER

Ludwig-Maximilians-University

A comparative study of neural differentiation in primates

Linking genetic to phenotypic changes is an essential, yet highly challenging task in biology. The evolutionary perspective can shed light on this relationship through the traces that adaptation and constraint have left in different biological systems. Hence, a comparative approach enables us to identify rules of regulatory changes and help to differentiate between important and more spurious links and nodes in regulatory networks. However, investigating early embryonic developmental processes in humans and our closest relatives, non-human primates, is challenging, not only because of the difficult acquisition of the primary material, but also because of substantiated ethical concerns. Induced pluripotent stem cells from a wide range of primates have the potential to close this gap, as they can be differentiated to almost any desired cell type and therefore mimic early developmental processesin vitro. Here we have utilized iPSCs of human, gorilla and cynomolgus macaques to investigate the neural induction process using single cell transcriptomics. We analyzed cell compositions, reconstructed the differentiation trajectories and compared these between the species. We found that cynomolgus cells differentiated at a faster rate than human and gorilla. To account for these differences we ordered the cells along a common pseudotime and identified a set of differentially expressed genes between early and late differentiation stages shared across species. Furthermore, we found that genes that are constantly upregulated along the differentiation trajectory are significantly enriched for transcriptional regulators. Using this information we identify transcriptional regulators whose expression is conserved in all three species, and that are therefore essential for early neural differentiation. With this approach we demonstrate the usefulness and strength of the evolutionary perspective when investigating dynamic processes to identify conserved patterns of gene expression and regulation.

DAVID GOMEZ JIMENEZ

NanoString Technologies

"Profiling antigen presentation through single-cell RNA-sequencing analysis of virally induced head and neck cancers"

Tonsillar cancer (TC) is a subset of head and neck malignancies associated to a high prevalence of human papilloma virus (HPV) infection (> 90% in Sweden). Due to the presence of viral antigens and its proximity to lymphoid tissue, TC stands as a candidate to immunotherapeutic treatment. Therapies targeting antigen presenting cells are of key importance to generate a diverse, yet sustained antigen-specific cell mediated immunity (Jimenez D., et al. 2021. Cancers; Askmyr D., et al. 2021. Human Immunology). In this study, we investigate the cellular diversity of antigen presenting cells (APC) in TC to resolve their function and identify unique molecular targets.

We have sorted over 10K single myeloid APCs from 5 HPV+ TC biopsies and a paired healthy control. We have characterized their transcriptome using single-cell RNA sequencing and identified 8 populations of APCs. Differential gene expression analysis has revealed unique patterns of immune checkpoint gene expression of these APC populations. In turn, gene-set variation analysis highlights complementary, yet specialized-subset functions e.g., phagocytosis, antigen presentation of exogeneous peptide though MHC-I and MHC-II, positive regulation of T-cell and granulocyte chemotaxis.

Poster session

We have also investigated the distribution of these populations in tissue using multiparameter flow cytometry and immunohistochemistry.

We have observed a 3-fold-increase of total myeloid APCs in the tumor microenvironment, and specific interactions of these 8 APC populations with T-cell subsets. The interaction of two cell types is defined by their expression of complementary surface receptors and ligands. To this end, we have characterized a publicly available single cell dataset of 22K single immune cells from 3 TC and 5 healthy tonsils and merged it with the myeloid APC dataset. We have performed receptor-ligand analysis and we have defined unique cytokine and chemokine axes that are involved in the crosstalk and priming of T-cell subsets. To investigate the clinical relevance of the 8 APC populations, we have extracted gene signatures and used them to score and stratify patients with the objective of identifying TC patients that would benefit from immunotherapy.
In summary, we have identified 8 distinct tumor infiltrating APC populations which differ from those seen in healthy tissue in frequency and function. This step is crucial in the way of designing new immunotherapeutic approaches that could reduce treatment side effects and alleviate societal costs of €117 512 per TC patient (Silfverschiöld M., et al. 2019. PlosOne).

Poster session

CARMEN GOMEZ ESCOLAR

"Profiling antigen presentation through single-cell RNA-sequencing analysis of virally induced head and neck cancers"

Germinal centers (GC) are microstructures where B cells that have been activated by antigen can improve the affinity of their B cell receptors and differentiate into memory B cells (MBCs) or antibody secreting plasma cells. Activation Induced Deaminase (AID) initiates antibody diversification in GCs by somatic hypermutation and class switch recombination. Here we have addressed the role of AID in the terminal differentiation of GC B cells by combining single cell transcriptome and immunoglobulin clonal analysis in a mouse model that traces AID-experienced cells. We identified 8 transcriptional clusters that include dark zone and light zone GC subsets, plasmablasts/plasma cells (PB), 4 subsets of MBCs and a novel prePB subset, which shares the strongest clonal relationships with PBs. Mice lacking AID have various alterations in the size and expression profiles of these transcriptional clusters. We find that AID deficiency leads to a reduced proportion of prePB cells and severely impairs transitions between the prePB and the PB subsets. Thus, AID shapes the differentiation fate of GC B cells by enabling PB generation from a prePB state.

Poster session

PABLO IÁÑEZ-PICAZO

Josep Carreras Leukemia Research Institute

DeepSCore: A multi-platform deep learning tool for the automatic cell-type annotations of single-cell multi-omics data

Data annotation and cell-type recovery is a crucial step in any standard single-cell data analysis pipeline. Deep Learning (DL) models conveniently enable the annotation of a dataset using another reference dataset that contains the information to transfer. Here, we present DeepSCore, a DL tool for automatic label transfer of any kind of cell-related information between single-cell datasets. With scRNA datasets, DeepSCore is trained using genes as features.With scATAC datasets, DeepSCore relieves the need to infer a noisy gene activity matrix by directly learning the information encoded in genomic regions. The model can be employed in both R and Python, and effectively achieves accuracies higher than 93% inboth data modalities with enough data. While the model shows high performance in most scenarios, the user can flexibly and easily modify the base architecture of the model to its specific needs. Furthermore, DeepSCore source code also includes multiple utility functions to facilitate single-cell data manipulation, such as finding a common set of peaks to merge scATAC datasets, filtering features or comparing clustering and annotation accuracy by plotting a Jaccard

similarity matrix. The application will besoon publicly available at https://github.com/pabloswfly, and will be released along with Jupyter Notebook tutorials. We envision the usage of DeepSCore as a user-friendlytool to facilitate universal single-cell data annotations and promote interoperability between R and Python existing pipelines. This is especially imperative in Python, where tools for scATAC data analysis are lacking. Additionally, we anticipate the inclusion of functionalities for the integration with spatial transcriptomic datasets inthe close future. Our tool will therefore represent a unified and consistent reference-based framework for the cell annotation of multimodal data.

Poster session

SUZANNE JIN

Centre for Genomic Regulation

Towards a composition-aware analysis of single-cell data

Most gene expression data are compositional1,2due to a sequencing artefact known as the “constant-sum constraint”. Consequently, the expression of a gene in different samples cannot be compared directly but only with respect to an internal reference (usually a normalization). Here we aim to better understand repercussions for single-cell co-expression inference in scenarios where normalization assumptions break down.We use both synthetic and experimental data to create a framework in which we can exactly determine the compositional bias and then study how it affects gene association measures. With the simulations3, we create samples with varying mRNA content that preserve the gene correlations. For the experimental set-up, we use scRNA-seq data collected from an asynchronous population of cells thatexhibit mRNA total changes by a factor of two along the cell cycle4. Since in our case a cell-cycle stage comes along with a known mRNA total, this dataset can be used as the ground truth internal reference. Not knowing this reference leads to considerable distortions –a plausible scenario for relative gene expression data5. With this set-up, we can compare the amount of co-expression determined on relative (biased) data

against the reference. We benchmark the performance of simple association measures(like correlation and proportionality1,6) in combination with different normalizations, log-ratio transformations, regularization techniques, and zero-replacement strategies. Our preliminary results confirm what theoretical considerations7suggest: while measures like Pearson correlation in combination with off-the-shelf normalizations can fail spectacularly, partial correlations on log-ratio transformed data are the most robust. Additional observations also provide insights on how to improve covariance regularization and shrinkage estimates for compositional data. Our work will lead to new tools and recommendations for researchers aiming to infer gene co-occurrences from single-cell data.

Poster session

ANOUSHKA JOGLEKAR

Weill Cornell Medicine

Towards a composition-aware analysis of single-cell data

Complex systems such as the brainleverage alternative splicing to expand the proteome. To enable splicing studies in distinct cell typesof these organs, we developed ScISOr-Seq, which identifiesfull-length isoforms from single cells1. We furthermore developed algorithmsto quantify and test differential isoform expression between cell-types, usingcomplete isoforms as opposed to single exons,TSS,or polyA-sites.We showed that isoforms of Fgf13display distinct colocalization patterns within pyramidal neurons and interneurons in the hippocampus,consistent with their known function. We demonstrated that regional identity can sometimes, although rarely, override cell-type specificity. Additionally, cell types indigenous to one anatomic structure show uniqueisoform expression patterns. Thus, we developed slide-isoform sequencing (Sl-ISO-Seq) to yield a spatially resolved splicing map in the developing mouse brain. Application of Sl-ISO-Seq revealed splicing patterns ofdevelopmentally regulatedgenes such as Snap25whichdisplay regionalgradients of expression throughoutthe brain2.In addition to these results, I will furthermore comment on results currently under review, revealing high cell-type specific usage

of exonsrelated to autism spectrum disorder, but not amyotrophic lateral sclerosis,in the medial frontal cortex.This justifies cell-type specific investigation of splicing, TSS and polyA-site usage in neurological diseases3.Last but not least, I will describe current efforts within the NIH Brain Initiative to study splicingacross multiple brain regions and developmental time points, revealing the broad patterns and exceptions of cell-type specific splicing patterns4.Taken together, I will present a comprehensive view of regional and temporally mediatedpatternsof splicingin the brain.

Poster session

PANAGIOTIS KARRAS

VIB Center for Cancer Biology, KU Leuven

Perivascular niches dictate developmental cellular hierarchies during melanoma growth

Cellular plasticity endows cancercells with capacitytowards a phenotypic state. Except from the genetic origin of cellular plasticity, cancer cells can transit to reversible phenotypic states as a result of microenvironmental cues and/or treatment-imposed pressures and are often driven by stochastic epigenetic and/or transcriptional fluctuations. Melanoma is a prime example of heterogeneous and plastic tumors where the origin and magnitude of cell state diversity remains poorly understood.Combining multicolor lineage tracing, 3D imaging and quantitative mathematical modelling in a clinically-relevant mouse model of melanoma that develops spontaneous tumors, we demonstratedthat tumors follow a hierarchical model of growthsupported by a population ofMelanoma Stem-like Cells (MSCs)that exhibit a transcriptomic signature of pre-migratory neural crest cells established transiently during embryonic development. ScRNA seq data inmouse and drug naïve human biopsies revealed that MSCs are evolutionarily conservedand independent of the “genetic makeup”of the tumors. Trajectory inference approaches further supported that MSCs are sitting at the top this hierarchy-givingrise to multiple lineages during progression. By deploying

unsupervised and targeted spatial transcriptomic approaches together with multiplex imaging, we developed a spatially and temporally resolved map of the diversity of melanoma cell states showing a non-random tumor organization. Multimodal analysis unraveled unique cell type andstate interactions and importantly demonstrated that MSCs reside inperivascular niches favoring tumor growth. Endothelial Cells were found to have a key role in the acquisition of stemness properties. Co-culture assays at 2D and 3D resolutionled to melanoma dedifferentiation and proliferation advantage. Of note, supplementing melanoma cells with ECs accelerated the onset and growth of tumors in vivo, where MSCs pool was significantly increased.Taken together, these results will pave the way for the development of strategies thatexploitthe “chameleonic”nature of cancer cells and, ultimately, target MSC niche-dependent specificationmechanisms.

Poster session

ALEX LEDERER

École Polytechnique Fédérale de Lausanne

Molecular profiling of stem cell-derived retinal pigment epithelial cell differentiation established for clinical translation

Human embryonic stem cell-derived retinal pigment epithelial cells (hESC-RPE) are a promising cell source to treat age-related macular degeneration (AMD). Despite several ongoing clinical studies, a detailed mapping of transient cellular states during in vitrodifferentiation has not been performed. Theavailability of single-cell RNA sequencing thusrepresents a compelling opportunityto systematically phenotype intermediate differentiation populations.Here we conduct transcriptomic profiling of a hESC-RPE monolayer protocol that has been developed for clinical use.Differentiation progressed through an unexpected culture diversification recapitulating early embryonic development, in which cells rapidly acquired a rostral embryo patterning signature, before converging towards the RPE lineage, as benchmarked to embryonic and adult retinal tissue references. At intermediate steps, we examined the potency of a NCAM1+ progenitor population and showed protocol's abilityto suppress non-RPE fates. Ourprocedure was robust across three cell lines and more rapidly specifiedRPE thana comparable 3D embryoid body protocol. We demonstrated that the method produces a pure RPE pool capableof maturing further after subretinal transplantation intoalbino rabbits. Our evaluation of hESC-RPE differentiation supports the development of safe and efficient pluripotentstem cell-based therapies for AMD.

Poster session

LEIF LUDWIG

Cellular states, purifying selection, and clonal dynamics in Pearson syndrome revealed via single-cell multi-omics

Alterations in mitochondrial DNA (mtDNA)are associated with a spectrum of often multi-systemic disorders, but also cell-type specific defects. Large deletions in mtDNA have been linked to a variety of clinical pathologies, including newly arisingin congenital disorders such as Pearson syndrome(MIM:557000), a mitochondrial disease characterized by sideroblastic anemia and exocrine pancreas dysfunction. Here, we establish a multi-omics approach to quantify mtDNA deletion heteroplasmy and features of cell state in thousands of single cells. By profiling primary hematopoietic cells from three patients with Pearson syndrome, we observe widespread transcriptional changes and altered AP-1 transcription factor activity. We resolve the interdependence between pathogenic mtDNA heteroplasmy and cell lineage, including purifying selection against mtDNA deletions in effector-memory CD8 T cell populations,further suggesting cell state specific metabolic vulnerabilities. Additionally, single-cell analyses of in vivoand in vitrodifferentiated bone marrow mononuclear cells reveal multi-faceted clonal dynamics in a patient with both Pearson syndrome and del7q myelodysplastic syndrome (MDS). Our results identify genomic perturbations underlying this mitochondrial disorder and more generally provide a multi-omicsframework to the study of human disease evolution at single-cell resolution.

Poster session

ETTORE LUZI

Laboratory of ncRNAs Complex Diseases University of Firenze

RISC-mediated control of selected chromatin regulators stabilizes ground state pluripotency of mouse embryonic stem cells

Background: Embryonic stem cells are intrinsically unstable and differentiate spontaneously if they are not shielded from external stimuli. Although the nature of such instability is still controversial, growing evidence suggests that protein translation control may play a crucial role.

Results: We performed an integrated analysis of RNA and proteins at the transition between naïve embryonic stem cells and cells primed to differentiate. During this transition, mRNAs coding for chromatin regulators are specifically released from translational inhibition mediated by RNA-induced silencing complex (RISC). This suggests that, prior to differentiation, the propensity of embryonic stem cells to change their epigenetic status is hampered by RNA interference. The expression of these chromatin regulators is reinstated following acute inactivation of RISC and it correlates with loss of stemness markers and activation of early cell differentiation markers in treated embryonic stem cells.

Conclusions: If this suggests a primary mechanism of miRNAs in preserving ES cell pluripotency and inhibiting the onset of embryonic differentiation programs, on the other hand miRNA- mediated control of chromatin regulators might maintain cells in a metastable state, which could rapidly be converted into priming to cell differentiation upon the removal of stemness-sustaining factors. Accordingly, priming could be seen as a process in which two epigenetic mechanisms are layered one on the other :a first layer, which is microRNA-mediated, on which lies a second layer consisting of chromatin-based modulation of transcription.Single-cell transcriptomics can help to study the transition states and cell fate decision inthese “Waddington epigenetic landascapes”.

Poster session

IKENNA C. MADUAKO

Glypican-3 dependent protein regulation in hepatocellular carcinoma and the potential role in cancer chemoprevention

Background:Hepatocellular carcinoma (HCC) is one of the world's lethal cancer and thirdamong all cancer-related mortalities. With staggering incidence in Asia and Sub-Saharan Africa. Natural dietary products have shown strong promising potentials in the management and prevention of cancer. [6]-gingerol (1-[4′-hydroxy-3′-methoxyphenyl]-5-hydroxy-3-decanone), a phenolic compound from ginger (Zingiber officinale) possesses chemoprotective properties against hepatocarcinogenesis in animal models. This study aimed at investigating the molecular mechanisms underlying the chemotherapeutic potentials of [6]-gingerol on diethylnitrosamine initiated and 2-acetylaminofluorene promoted hepatocellular carcinoma in mice.

Methods: Forty male BALB/c mice (15 –18 g) were distributed into four groups of ten animals each. Group 1: were treated with corn oil. Group 2: received single i.p dose of DEN (75 mg/kg) in normal saline after 7 days followed by 2-acetylaminofluorene (200 mg/kg) 2-AAF in diet. Group 3: was treated with 6-GR (100 mg/kg) only. Group 4: Mice were pre-treated with [6] gingerol in corn oil for 7 days and thereafter treated with the same treatment regimen as obtained in group 2, treatment continuedfor 24 weeks.

Results: Inflammation was evaluated by measuring the levels of IL-1β, IL-6, TNF-α, and the expressions of NF-κβ, and COX-2. We observed that [6]-gingerol inhibited HCC-induced activation of wnt signaling pathway and exerted anticancer activity via abrogation of glypican-3, c-Myc, TGF-β, HIF and VEGF-A expressions.

Conclusion: With strong evidence, 6-GR exhibited potent anticancer potentials against DEN and 2AAF-induced hepatocellular carcinoma by inhibiting glypican-3 pathway and associated proteins.

Impact: Based on our findings, 6-GR provided natural alternative therapy against HCC.

Poster session

RAMON MASSONI-BADOSA

The periodic table of tonsillar cells

Secondary lymphoid organs (SLO) are essential to develop tolerance and adaptive immunity against self or foreign antigens, respectively. Of note, palatine tonsils (tonsils hereafter) are commonly used as model SLO, as they are easily accessible through routine tonsillectomies. Tonsils are located strategically at the intersection between the respiratory and digestive tracts, where they are bombarded with antigens. Although some studies have performed scRNA-seq on human tonsils, they focus on specific cell populations, use a single layer of information, or lack the number of cells necessary to identify rare cell types. Hence, a comprehensive compendium of cell types and states in a human SLO is still lacking. Here, we profiled over 350,000 cells from ten tonsils with four techniques (scRNA-seq, scATAC-seq, 10X multiome and CITE-seq. In addition, we performed spatial transcriptomics (10X Visium) to map the exact location of each cell type state. We identified a new superenhancer that regulates BCL6 in T follicular helper cell differentiation, new subsets of SLAN+ macrophages, and a perivascular precursor follicular dendritic cells, which was previously uncharacterized in humans. In addition, we developed SLOcatoR, an R package that allows the user to install and explore the tonsil with few lines of code. Projecting mantle cell lymphoma cells (MCL) from two diseased tonsils with SLOcatoR identified compositional shifts in the tumor microenvironment, with marked enrichment of T regulatory cells. In conclusion, by combining multiple layers we obtained a holistic view of tonsillar cells identity, yielding a high-quality cell atlas. This atlas is a robust platform for hypothesis generation and mapping of unseen transcriptomes or chromatin accessibility profiles derived from SLO

Poster session

SARA MONTSERRAT

Bellvitge Institute for Biomedical Research (IDIBELL),

Targeting CDC42 activity in vivorejuvenates the transcriptome of aged hematopoietic stem cells

The ability to restore or rejuvenate aged tissues by targeting stem cells is a central goal of regenerative medicine. In the hematopoietic system, treating aged hematopoietic stem cells (HSC) ex vivowith a Cdc42 activity-specific inhibitor (CASIN) restored the cell polarity and the epigenetic asymmetry of HSC divisions that was lost with age, while a systemic treatment with CASIN on aged mice significantly extended their average lifespan. We now explored whether the systemic CASIN treatment affects aged HSCs phenotype and function. After experimentally confirming an increased cell polarity and regenerative capacity in the HSCs of aged CASIN-treated mice, we performed single cell RNA sequencing profiling of bone marrow hematopoietic stem and progenitor cells (Lin-c-Kit+ Sca1+ cells) of these mice, as well as young and aged control mice, analyzing a total of 15,856 cells that divided into 13 clusters. We observed an increased frequency of HSCs in the aged bone marrows that was not reduced after CASIN treatment, as also previously demonstrated experimentally. However, CASIN significantly downregulated the expression of several genes involved in quiescence and stress response, which were highly expressed in non-treated aged HSCs. We further observed a decrease with age in the connectivity between the different HSC clusters and between HSCs and multipotent

progenitors that was partially restored in the bone marrow cells of aged CASIN-treated mice. The analysis of gene expression levels along trajectories between pairs of clusters revealed changes in the pattern of expression of genes involved in myeloid and lymphoid differentiation and the response to transforming growth factor βin aged cells after CASIN treatment. Overall, these results support that systemicCASIN treatment of old mice for 4 consecutive days modifies the transcriptome of aged HSCs, which show a rejuvenated molecular profile supporting the improvement in their regenerative capacity.

Poster session

GEMMA NOVIELLO

CasTuners: a degron- and Cas- proteins based toolkit to finely tune gene expression at the single-cell level

Not only the presence or absence of certain gene products dictate cell states but also their specific quantities or dosage. One example is the up-regulation of Xist, the master regulator of X-chromosome inactivation, which is induced only in female cells through a double dose of X chromosomal genes. Interrogation of dosage-sensitive gene regulation requires experimental tools to precisely tune activity of transcriptional regulators. However, most of the currently employed approaches to manipulate gene expression, rely on the strong up- or down-regulation of a target gene or are labour intensive.

We developed CasTuners: an easy-to-use degron- and Cas- proteins based toolkit to quantitatively control gene expression at the single cell-level. We employ catalytically dead Cas9 (dCas9) fused with a repressor domain or CasRx, to control gene expression at the transcriptional or post-transcriptional level, respectively. We systematically compared several degron domains and selected the one allowing the tightest regulation of our Cas proteins. We exploited an endogenous reporter system in mouse embryonic stem cells to compare the effects of different repressor mechanisms and showed that histone deacetylation but not H3K9 methylation can tune gene expression at the epigenetic level.

We therefore developed two systems (HDAC-dCas9 and CasRx) that allows to finely tune gene expression in single cells.

Poster session

GIULIA PROTTI

Single-Cell Analysis Revealed Transcriptional Response of Dendritic Cell Subsets During SARS-CoV-2 Infection

Human conventional dendritic cells (cDCs) are a heterogeneous population originally classified in cDC1s and cDC2s, based on developmental and functional criteria. Recent advances in single-cell technologies have improved cDC classification by identifying novel subsets, such as the newly identified DC3s, characterized by the expression of both cDC2 marker genes and genes classically associated with monocytes [1]. Although cDCs constitute a rare immune cell population in peripheral blood, growing evidence suggests that these cells undergo aberrant maturation during SARS-CoV-2 infection and this negatively affects T cell activation. Given the pivotal role of cDCs in the orchestration of adaptive immune responses, a deep characterization of their transcriptional signatures during COVID-19 may provide novel insights to understand the immune system's reaction to infection.

Here, we analysed two available and a newly generated single-cell transcriptomic datasets of peripheral blood mononuclear cells from COVID-19 patients and healthy donors (HD). Interestingly, during infection, DC3s showed increased frequencies in patients, which positively correlated with disease severity. When comparing cDCs in severe versus mild patients, we identified an important number of differentially expressed genes. Specifically, inflammatory genes not related to the activation of adaptive immunity, like complement and 1

coagulation factors, were upregulated in cDC2s from severe patients. Conversely, genes encoding MHCII molecules, the costimulatory molecule CD86 and cytokines, showed a progressive downregulation from HD to mild and finally severe patients. Hence, as disease severity increases, cDC2s progressively skew toward inflammatory activities and lose the antigen presenting function.

In conclusion, we unveiled the transcriptional signatures, reflecting the functional state, of cDC subsets during COVID-19. Importantly, by inducing the downregulation of crucial molecules required for T cell activation, the virus implements an efficient immune escape mechanism that correlates with disease severity.

References [1] Villani et al. Science 20

Poster session

PAU PUIGDEVALL

UCL Great Ormond Street of Child Health, University College London

Effects of somatic and engineered mutations on cellular phenotypes in iPSC models of differentiation

The use of induced pluripotent stem cells (iPSC) as models for development and human disease has enabled the study of otherwise inaccessible tissues. Aremaining challenge in developing reliable modelsis ourlimited understandingof the factors driving irregular in vitrodifferentiationof iPSCs, particularly the impact of acquired somatic mutations. We leveraged data from a pooled dopaminergic neuron differentiation experiment of 238 iPSC lines profiled with single-cell sequencingand whole-exome sequencingto study how somatic mutations affect differentiation outcomes.Differentiation was tracked at three time points corresponding toneural progenitors, early neurons and mature neurons. We found that deleterious somatic mutations in key developmental genes, notably the BCOR gene, are strongly associated with failure in dopaminergic neuron differentiation, with lines carrying such mutations also showing larger proliferation rate in culture. We further identified broad differences in cell type composition between failed and successfully differentiatinglines, as well as significant changes in gene expressioncontributing tothe inhibition of neurogenesis, afunctional process also targeted by deleterious mutations in failed lines. Some of the cellular phenotypes observed among lines that failed differentiation were also observed in lines with engineered knockoutsof known developmental disorder genes, namely ASXL3and CTNNB1. Our work highlightsthe need to routinely measure the deleterious somatic burden of iPSC linesand calls for caution ininterpretingdifferentiation-related phenotypesindisease-modelling experiments.

Poster session

CRISTINA RIOBELLO SUÁREZ

Gene Regulatory Control in Disease, CiMUS, USC, Santiago de Compostela

Metabolic adaptation as an essential requirement for liver regeneration

In clinical situations, the survival of the patient after tumorresection or after orthotopic liver transplantation is clearly determined by the ischemic damage suffered by the organ during the surgery and by its intrinsic capacity to regenerate. The capacity to regenerate after injury is dependent on a coordinated regulation of metabolism and hepatocyte division during liver renewal, since malfunction of these processes usually leads to liver failure. For instances, metabolic alterations, including hepatic steatosis and NASH, have been associated with increased complications and post-operative mortality after major liver resection. Although liver regeneration has been studied extensively at many levels, an analysis of the metabolic requirements for hepatocyte proliferation during liver regeneration in vivohas not beenstudied in depth. In this work, we have constructed a map of the cellular landscape of the regenerating liver, under normal and pathological situations, using single cell RNA sequencing and computational approaches. Altogether, our study providesa comprehensive view of the regenerating liver at single-cell resolution of differential populations of cellsin normal and steatotic livers.

Poster session

AIDA RIPOLL-CLADELLAS

Barcelona Supercomputing Cente

Single-cell transcriptomics reveals insights into telomere shortening with aging

Although aging is a universal process affecting all tissues, the underlying cellular and molecular transcriptional mechanisms remain largely unknown. Telomere length has been proposed as one of the main hallmarks of aging. The average length of telomere repeats declines with age in cells of most self-renewing tissues. However, the interconnectedness between telomere length and its contribution to aging at the cell-type resolution level is not yet well understood. Here, we studied the relationship between telomere length changes and gene expression at single-cell resolution in 62 donors from the Netherlands Lifelines Deep cohort. We coupled clinically validated flow-FISH measurements of telomere length in six blood cell types to the expression level of corresponding cell types using single-cell RNA-sequencing data. This revealed 97 genes whose expression level varied with changes in telomere length in T cells. Three of them (DNAJA1, EEF1A1, RPL29) were previously reported as telomere binding proteins, indicating that our approach captures genes directly involved in telomere length dynamics. Moreover, the genes negatively associated with telomere length were enriched for pathways related to translation and nonsense-mediated decay, which might have important physiological

consequences. Even though some of the telomere length-associated genes were located near the telomere ends, in general, our differential expression findings could not be explained by previously described mechanisms (telomere position effect (TPE)or TPE over long distances (TPE-OLD)). It suggests that T cells are sensitive to telomere length-induced expression changes that can act through both short-and longer-range interactions, but also that cell expression may directly influence telomere dynamics. Altogether, this study reveals the importance of further exploring the context-specificity of telomere shortening-induced changes that occur with aging or specific treatments.

Poster session

MARINA RUIZ-ROMERO

Centre for Genomic Regulation (CRG)

scFLEA-ChIP: deciphering the epigenetic signature of development and cancer at single cell level

All pluricellular organisms develop from a single totipotent cell. In the course of development, cells differentiate and get committed to distinct cell fates to, ultimately, form specialized tissues. Through these processes, cells accumulate epigenetic changes, such as DNA methylation and histone marks, leading to high epigenetic diversity within cell populations. Uncovering the epigenetic changes occurring along dynamic processes at the single cell resolution will foster the understanding of the establishment of cell lineages during development and differentiation, as well as other processes, such as cancer progression, and will allow to decipher how cellular heterogeneity contributes to different outcomes along these processes. However, the lack of a reproducible and endorsed protocol to identify protein-DNA interactions at single-cell level represents an important constraint in the field. Aiming to uncover cell heterogeneity from the epigenomic perspective and to provide the scientific community with an affordable single-cell ChIP-Seq protocol, we have recently developed single-cellFLEA-ChIP, a methodology to identify protein-DNA interactions at the single cell level. scFLEA-ChIP can be adopted by any Molecular Biology lab as it does not require the usage of specific devices or complex steps.

Poster session

It relies on cell sorting technologies to isolate and barcode individual cells prior to immunoprecipitation, reducing to the minimum cell remnants and undesired nucleic acids in suspension that could jeopardize the identification of single cell profiles. The scFLEA-ChIP also incorporates an innovative approach to efficiently amplify ultra-low input libraries that prevents the generation of thousands of a few PCR duplicates, avoiding bias derived from successive in-vitro transcription and retrotranscription rounds. Using this method, we have performed H3K4me3 scFLEAChIP assays from as few as 1,500 culture cells, obtaining chromatin profiles comparable to bulk ChIP-Seq experiments performed with a similar number of cells. We are currently applying this technology to delineate the epigenetic profile of individual cells from differentiation and cancer models to uncover cell heterogeneity and to decipher the relationship between different cell subpopulations

Poster session

PABLO SACRISTÁN-GÓMEZ

instituto Investigación Sanitaria La Princesa

Spatial transcriptomics to unravel new roles of thyrocytes and immune complexity in Hashimoto´s Thyroiditis.

INTRODUCTION: Hashimoto´s Thyroiditis (HT) is an autoimmune thyroid disease characterized by thyroid follicles destruction and autoantibodies against thyroid antigens. Itis evident that genetics (SNPs), epigenetics (HDACs/HATs),environmental factors (age, sex...)and impaired function of several immune cells are involved in its development. However, HT pathogenesis has not yet been fully elucidated.

OBJECTIVE: Determine different transcriptome profiles of specific immune and thyrocytes subpopulations associated to HT development.

METHODS: 3 HT and 2healthy controls were analyzed using Visium Spatial Gene Expression. Data from each condition was integrated in order to compare between thyrocytes, immune and connective tissue clusters. Each sample was visualized and analysed using Loupe Browser. Cluster annotation was performed by pathological characteristics (H&E) and transcriptomic databases (xCell). Functional enrichment analysis was assessed by GO and KEGG databases. Putative genes were analysed using Loupe Browser per sample.

Poster session

RESULTS: Integrative data from HT patients revealed specific clusters that belonged to damaged thyrocytes, immune subpopulations like follicular dendritic cells located in germinal centres, neutrophils/monocytes, memory T cells, B cells/plasma blasts and connective tissue. Germinal centres presented a highly increase of HMGB1, FCγRIIIb related to Neutrophil Extracellular Traps (NETs). Transcriptomic profiles of HT in comparison to controls showed different subsets of thyrocytes at metabolism level; and alterations in the connective tissue related to ECMorganization.

CONCLUSION: Using spatial transcriptomics approach, we have characterized the transcriptomic heterogeneity and regulatory networks within cell types in thyrocytes, connective tissue and infiltrating lymphocytes, discerningn ovel potential pathways, not seen previously at tissue level, related to HT pathogenesis.

NURIA SÁNCHEZ DE LA BLANCA

instituto Investigación Sanitaria La Princesa

Integration of spatial transcriptomics (ST) data from different sampleconditions

Introduction: Visium 10x Genomics ST is a relative recent technique where mRNA is sequenced insitu in spots from a sample slide. It supposes an opportunity of developing new bioinformaticprotocols adding the spatial information. The benefits of this strategy could be applied inautoimmune diseases such as Hashimoto's thyroiditis (HT) to explore the perplexing roles of thedifferent cell subsets and understand the causal mechanisms of immune tolerance in these diseases.

Objective: To evaluate a bioinformatic ST pipeline in thyroid tissue of HT patients compared withcontrols. To determine potential biomarkers and pathways implicated in the pathogenesis of thedisease in the different cell subsets, such as thyrocytes, immune cells, or tissue microenvironment.

Methods: Three HT samples and two tissue controls were processed using Visium approach. Eachsample was analyzed individually and then integrated by condition aiming to explore the ability ofSeurat R package clusters at different resolutions and consensus through the mentioned samples.Heterogeneous areas were reclusterized to obtain minimal subpopulations. Then, cell populationswere annotated following two

strategies: automatic packages (XCell, UCell) and manually namingcell subgroups using coexpressed markers on the spots already described. Additionally,characteristic gene groups and metabolic pathways in clusters were obtained (GSEA, KEGG,Reactome) and evaluated.

Results: Bioinformatic pipeline was consistent with the wet-lab experiments and bibliography. Wewere able to obtain genes and cell populations HT specific and metabolic pathways that could playimportant roles in its pathogenesis.

Conclusion: ST can be used to understand and explore diverse pathophysiological mechanismsunderlying autoimmune disorders.

Poster session

NURIA SÁNCHEZ DE LA BLANCA

Barcelona Supercomputing Center

Invivo single-cell profiling of lncRNAs during Ebola virus infection

Long non-coding RNAs (lncRNAs) are pivotal mediators of systemic immune response to viral infectionyet most studies concerning their expression and functions upon immune stimulation are limited to in vitrobulk cell populations. This strongly constrains our understanding of how lncRNA expression varies at single-cell resolution, and whether differences with protein-coding genes exist in their cell-type specific immune regulatory roles. Here, we perform the first in-depth characterization of lncRNA expression variation at single-cell resolution during Ebola virus (EBOV) infection in vivo. Using bulk RNA-sequencing from 119 samples and 12 tissue types, we significantly expand the current macaque lncRNA annotation. We then profile lncRNA expression variation in immune circulating single-cells during EBOV infection and find that lncRNAs' expression in fewer cells is a major differentiating factor to their protein-coding gene counterparts. Upon EBOV infection, lncRNAs present dynamic and mostly cell-type specific changes in their expression profiles especially in monocytes, the main cell type targeted by EBOV. Such changes are associated with gene regulatory modules related to important innate immune responses such as interferon response and purine metabolism. Within infected cells, several lncRNAs have correlated and anti-correlated expression with viral load, suggesting that expression of some of these lncRNAs might be directly hijacked by EBOV to attack host cells. This study provides novel insights on the roles that lncRNAs play in the host response to acute viral infection and paves the way for future lncRNA studies at single-cell

Poster session

RODRIGO SENOVILLA-GANZO

Achucarro Basque Center for Neuroscience

Conserved cell types in the early embryonic brain across vertebrates

Organogenesis is the most evolutionarily conserved developmental stage when comparing morphology and whole-body transcriptome across vertebrates. During this phylotypic stage, body axes are defined and cell types are given their spatial identity. Due to its physiological importance, these segmentation and patterning genes (e.g. HOXs) are thought to be evolutionary conserved. However, across vertebrates, there is yet no evidence of transcriptomic conservation in essential organs as the brain, nor identification of homologue neural cell types in its initial neurodevelopmental bauplan. Thus, by performing single cell RNAseq and in situ hybridization assays, we obtained cellular and molecular atlases of the early developing brains of five vertebrate species: chicken, gecko, mice, zebrafish, and human. These single cell atlases allowed us to identify the neuroanatomical identities that naturally segment different vertebrate early brains and the genes that pattern these regions. Secondly, to unbiasedly evaluate the transcriptional conservation of these cell types across species, we performed three complementary methods: correlation of gene specificity indexes, datasets integration (“RPCA”) and label transference. All approaches proved a high transcriptional conservation of equivalent morphogenic organizers and neuromeric identities among these vertebrate species, specially at transcription factor level. These results confirm the existence of a

common phylotypic brain as well as the conservation of homologue neural cell typesmastering its underlying bauplan. Therefore, this bauplan conservation is essential to stablish the foundations for assembling vertebrate brain structures, but also it sets the diversity boundaries within which these brains were allowed to evolve. Such an important constrain that early embryonic vertebrate brainhasbarely changed despite 500 million years of evolution.

Poster session

LUCAS WANGE

Ludwig-Maximilians-University

Prime-seq, efficient and powerful bulk RNA-sequencing

Cost-efficient library generation by early barcoding has been central in propeling single-cell RNA-sequencing.With more single cell atlases becoming available the interpretability and usefulness of bulk data increases and enables better experimental designs. Here, we optimize and validate prime-seq, an early barcoding bulk RNA-seq method. We show that it performs equivalent to TrueSeq, a standard bulk RNA-seq method, but is four-fold more cost-efficient due to almost 50-fold cheaper library costs. We also validate a direct RNA isolation step, show that intronic reads are derived from RNA, and compare cost-efficiencies of available protocols. We conclude that prime-seq is currently one of the best options to set up an early barcoding bulk RNA-seq protocol that complements single cell RNA-sequencing.