The 3rd International Symposium on Microgenomics 2023

Technical Workshop June 28

Symposium June 29 & 30, 2023

Learning Center Morgagni, University of Florence

Florence, Italy

Scan the code to download the program and abstract book:

Welcome to the International Symposium on Microgenomics

Dear Participants,

After an excessively long break, due in large part to the onset of the COVID-19 pandemic, we were finally able to organize this 3rd International Microgenomics Symposium, and it is a great pleasure, on behalf of the local organizing committee and scientific board of the conference, to welcome you to Florence. Similar to the second edition in Paris, the symposium will be preceded by a one-day Technical Workshop, on June 28th.

The focus of the 2023 symposium is:

New methodologies and strategies for rare and small sample analysis: from agriculture to medicine

The scientific program is organized into 4 sessions, each addressing specific themes:

1 - New Methodological Approaches and Instruments in Microgenomics Analysis
2 - Solid tissues and Spatial omics
3 - Circulating Biomarkers: Extracellular Vesicles & Single-Cells
4 - Integrative Analysis : from Multi-omics to Bioinformatics

The Symposium Talks, Poster Session, Industrial Exhibitions and associated Technical Workshops on Methods and Strategies for Molecular Biomarker Analysis, Spatial Transcriptomics & Single Cell, Extracellular Vesicles and Digital PCR & Microgenomics. These sessions aim to provide an overview of the present knowledge to obtain high quality molecules and future developments in Microgenomics analysis tools.

After the series of webinars organized in 2021, this is your chance to meet face-to-face with colleagues from around the world and across the scientific spectrum. This meeting is intended to be fully interactive, to build on existing collaborations, and to start new ones.

Around 100 scientists mainly from Europe and the USA will attend this third symposium. We are going to do our utmost to encourage scientific collaboration using every possible means. There will be various social events during the symposium providing opportunities for fruitful exchanges and creating good lasting memories. We invite you to join us for the gala dinner at the Grand Hotel Baglioni in their B-roof restaurant, where we will enjoy a delightful meal and a beautiful view of the city of Florence.

Posters will be displayed at each break and during lunches. During the scientific symposium, an Industrial Exhibition will take place. Eleven international companies will be presenting their newest services and technologies in the field. Our two GOLD sponsors, Bio-Rad and QIAGEN will each hold a lunch conference on Thursday and Friday, respectively.

The scientific and organizing committees of the Microgenomics 2023 Symposium express their warm thanks to the many contributors in the process who made the organization of the symposium possible. We extend our thanks to the editing committee and chairpersons, whose expertise was essential to the publication and discussion of the scientific contributions. We would also like to acknowledge our sponsors, whose support is essential to the achievement of this third symposium.

We are confident that all of you will have a productive meeting from a scientific point of view and that you will also enjoy the social events, landscapes, culture and hospitality in Florence.

We are very happy to welcome you this summer to Florence, during two days of highly professional, successful and enjoyable events.

Best regards,

Claudia Bevilacqua & Pamela Pinzani

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The Scientific Committee	The Organizing Committee
Claudia Bevilacqua	Claudia Bevilacqua
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WORKSHOP PROGRAMS & ABSTRACTS

Workshop 1 : Methods & Strategies for Molecular Biomarker Analyses

June 28, 9:00-13:00

Mikael Kubista

- Background and history
- qPCR – how to plan, optimize and validate
- dPCR – how to plan, optimize and validate
- NGS - how to plan, optimize and validate
- Protein analysis with NGS (profiling) and dPCR (ultrasensitive targeted)
- Preanalytics – How to identify the confounding step and how to optimize

Workshop 2 : Spatial Transcriptomics & Single Cell : June 28, 9:00-13:00

9:00-9:30	Single cell analysis: an overview, *Pamela Pinzani, University of Florence, Italy*
9:30-9:50	Single cell RNA sequencing and applications, *Letizia De Chiara, University of Florence, Italy*
9:50-10:10	Single Cell 3.0: From Bench to Insight, *Parse Biosciences*
10:10-10:30	*Spatial biology:* an overview, *Lance A. Liotta, Center for Applied Proteomics and Molecular Medicine, George Mason University, USA*
10:30-10:50	Laser microdissection: advantages and challenges for single cell applications, *Lance A. Liotta, Center for Applied Proteomics and Molecular Medicine, George Mason University, USA*
10:50-11:10	Coffee break
11:10-11:30	Targeted Spatial Molecular profiling for Precision Medicine. Molecular and Spatial Profiling of Individual cells, *Lance A. Liotta, Targeted Biosciences (Acculift Spatial Profiler)*
11:30-11:50	*Finding "The One": How microdissection and cell picking uncovers the hidden world of single cells,* Jörg Brühmann, Molecular Machines & Industries (MMI)
11:50-12:10	Explore new dimensions through spatial context, *Vizgen (Spatial Transcriptomics)*
12:10-12:45	From multitude to singularity: An up-to-date overview of scRNA-seq data generation and analysis, *Roberto Semeraro, University of Florence, Italy*
12:45-13:00	Final Q&A and Discussion

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Finding “The One”: How microdissection and cell picking uncovers the hidden world of single cells.

BRÜHMANN J

Abstract

In medical diagnostics or life science research, it is crucial to get high-quality homogeneous tissue samples, especially in genomics, transcriptomics, and proteomics. Enriched cell populations from tumor, endothelial tissue, histocytes, stem cells, and other materials are a prerequisite for analysis and patient profiling.

Laser microdissection is a widely used technology in life science research and for clinical applications for the accurate dissection of diseased cells from biopsy material. Thus, it is an essential tool for early diagnosis of cancers and neurological disorders, and it is established as a step towards personalized medicine. The MMI CellCut laser microdissection system combines proven cutting-edge technologies for the precise isolation of single cells and groups of cells.

[1] Molecular Machines & Industries (MMI), Breslauer Str. 2, 85386 Eching, Germany

Workshop 3 : Extracellular Vesicles : June 28, 14:00-18:00

14:00 *Introduction & MISEV guidelines*, **Michael Pfaffl** (moderation), TUM, School of Life Sciences, Freising, Germany

14:30 *Extracellular Vesicles - How to find the Needle in the Haystack*, **Christian Preusser**, EV Core Facility, Medical Faculty, University Marburg, Germany

15:00 *Convenient and Robust Isolation of EV RNA and Intact EVs*, **Martin Schlumpberger**, Qiagen R&D, Hilden, Germany

15:30 *Next Generation of NTA - Fluorescence Colocalisation Measurements*, **Christina Klasen**, Particle Metrix R&D, Inning am Ammersee, Germany

16:00 Coffee break

16:15 *EV Biomarker Discovery - Comparison of EV Isolation Methods, small-RNA Seq and RT-qPCR validation*, **Michael Pfaffl**, TUM, School of Life Sciences, Freising, Germany

16:45 *Digital PCR-Based Detection and Characterization of Extracellular Vesicle Biomarkers*, **Afif Abdel Nour & Ellen Bruske**, Qiagen R&D, Hilden, Germany

17:15 *caRNAge - comprehensive small RNA Seq data analysis*, **Benedikt Kirchner**, TUM, School of Life Sciences, Freising, Germany

17:45 Final Q&A and Discussion all participants

18:00 End & Cocktail Reception

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Next Generation of NTA - Fluorescence Colocalization Measurements

KLASEN C

Abstract

During the last decades, Nanoparticle Tracking Analysis (NTA) has emerged as a vital and fast characterization technology for biological nanoparticles like Extracellular Vesicles, Exosomes and Viruses. While classic NTA scatter operation feeds back particle size and total concentration, the fluorescence detection capability enables the user to gain specific biochemical information. Determination of biomarker colocalization however, is a challenging task for any analytical instrument. A new laser generation ensuring perfect overlap of the illumination volumes of individual channels paired with ultra fast switching times between fluorescence channels lay the foundation of colocalization nanoparticle tracking analysis (C-NTA) introduced recently by Particle Metrix. For the first time, we report results of colocalization measurements of reference material as well as real biological samples based on NTA technology on the new ZetaView®.

[1]Particle Metrix R&D, Inning am Ammersee, Germany

Workshop 4 : Digital PCR & Microgenomics : June 28, 14:00-18:00

14:00 - 14:50 *Principles of dPCR and the digital MIQE guidelines,* **Dr. Alexandra Whale**, Science Leader, Molecular Biology, National Measurement Laboratory at LGC, Teddington, *UK*

14:50 - 15:00 Gold sponsor presentation – BioRad, *Bio-Rad Droplet Digital™ PCR: for a multiplex of reasons*, **Dr. Lara Rossini**, ddPCR Regional Sales Specialist for Genomic - Southern Europe

15:00 - 15:15 Questions & Answers on general dPCR

15:15 -15:45 *Use of dPCR in GMO diagnostics*, **Dr. Alexandra Bogožalec Košir**, Scientific Associate, National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana, Slovenia

15:45 -16:15 *Digital PCR improves analysis of circulating cell-free miRNA in prostate cancer patients*, **Dr. Gerit Theil,** Head of the laboratory, Department of Urology and Transplantation, Martin-Luther-Universität Halle-Wittenberg

16:15 -16:30 Questions & Answers on applications of dPCR

16:30 -16:50 *Coffee break*

16:50 -17:00 Silver sponsor presentation – *Improving Gene Research Applications with Quant Studio Absolute Q dPCR*, **Monica Boita**, Senior Genetic Analysis Sales Specialist, Thermo Fisher Scientific

17:00 -17:45 *Data analysis and advanced assay design*, **Dr. Alexandra Whale**, Science Leader, Molecular Biology, National Measurement Laboratory at LGC, Teddington, UK

17:45 -18:00 Final Questions & Answers and closing remarks

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Digital PCR improves analysis of circulating cell-free miRNA in prostate cancer patients

BIALEK J, WEIGAND K, THEIL G

Introduction

Circulating, cell-free miRNA are considered as auxiliary biomarkers for early tumor development in prostate cancer (PCa) patients. Samples received from patients have small volumes, which limits the analysis possibility. Digital PCR is a sensitive technology providing absolute quantification, allowing to detect and analyze very low copy numbers of RNA due to fractionation of a sample into >20000 small compartment reactions.

Material and Methods

We analyzed serum levels of various miRNA, including miR-16 and miR-375 at different time-points, in a cohort of volunteers who participated in the 10-year screening study for PCa, performed by the University Clinic Halle/Saale, Clinic for Urology. Nineteen of them developed PCa, 15 - BPH and 19 - healthy men. For the analysis, we used Quant Studio Absloute Q Digital PCR System. We modified the manufacturer's protocol by adaptation of the conditions as described by Jiang et al., this included changes of time and temperature of single steps.

Results and Discussion

We detected increased levels (median; range) of miR-16 in PCa group (32768 cp/µL; 136-328528) comparing to BPH (4822 cp/µL; 148-91495 cp/µL) or healthy (18132 cp/µL; 0-35159 cp/µL) men. Expression of miR-375 was increased in the BPH group (584 cp/µL; 45-6598 cp/µL) compared to others (106 cp/µL; 0-2880 cp/µL – PCa group and 60 cp/µL; 0-341 cp/µL - healthy). Mach-paired analysis of the PCa group in various time points displayed decreasing expression tendency for miR-375. miR-16 expression decreased in the patients with a time distance longer than 1 year and increased for <1 year. The results displayed as absolute copy numbers/µL detect even weak expression like 0.11cp/µl show sensitivity of the method solving the problem of valid house-keeping reference. Considering these results, both miRNA can be proposed as PCa tumor suppressors.

Conclusions

dPCR is a method, which in a short-time and less working-steps improved the possibility of detection and analysis of miRNA as potential tumor markers in our cohort.

References

Jiang L., Lin R., Gallagher S., Zayac A., Butchbach M. E. R. and Hung P. 2020 "Development and validation of a 4 -color multiplexing spinal muscular atrophy (SMA) genotyping assay on a novel integrated digital PCR instrument." Scientific Reports Vol. 10 Issue 1 Pages 19892

[1] Medizinische Fakultät der Martin-Luther-Universität Halle-Wittenberg, Universitätsklinik und Poliklinik für Urologie

New methodologies and strategies for rare and small sample analysis: from agriculture to medicine

Thursday, June 29th 2023

8:00-9:00	Registration
9:00-9:10	Welcome : Alessandra Petrucci, Rector of the University of Florence

Session 1 New Methodological Approaches and Instruments in Microgenomics Analysis

Chairman: Claudia Bevilacqua & Alexandra Whale

9:10-9:40	Keynote lecturer: Mikael Kubista (Czech Republic) - *Spatiotemporal characterization of wound healing and regeneration in amphibians and of ischemic brain injury in mice*
9:40-10:10	Invited lecturer: Davide Cittaro (Italy) - *Sketching chromatin dynamics at single cell level*
10:10-10:40	Invited speaker: Viacheslav MYLKA (Belgium) - *Doing more with less: comparative analysis of sample multiplexing methods for single-cell RNA-seq*
10:40-10:55	Selected oral presentation: Nikoletta Galambos (France) - Young Scientist Grant - *Cellular specialization inside the symbiotic organ of an insect pest: deciphering the dialogue between host and symbiotic bacteria and their adaptation to different dietetic conditions*
10:55-11:25	Coffee break + Posters + Sponsors
11:25-11:55	Invited lecturer: Alexandra Whale (UK) - *Using digital PCR to characterize viral vector genomes*
11:55-12:15	1st Gold Sponsor: BIO-RAD, Sergey Yakushev (Switzerland) - *Examples of the duplex and multiplex ddPCR applications from the contract research organization practice*
12:15-12:30	Selected oral presentation: Eloise Debare & Benjamin Tisserand (France) - *OPTO-Cas9 allows easy, fast and precise spatiotemporal genome editing in vertebrate embryo at cellular resolution*
12:30-12:45	Selected oral presentation: Adele Calabri (Italy) - *Monitoring circulating tumor DNA during immunotherapy in Non-Small Cell Lung Cancer patients by targeted NGS: preliminary data on the CORELAB project*
12:45-13:00	Invited speaker: Fabio Morecchiato (Italy) - *dPCR vs. qRT-PCR for quantitative detection of SARS-CoV-2 RNA in environmental specimens*
13:00-14:30	Lunch + Posters + Sponsors: BIO-RAD

Session 2 Solid tissues and Spatial omics

Chairman : Pamela Pinzani & Mikael Kubista

14:30-15:00	Keynote lecturer: Pascal Barbry (France) - *Omics at the bedside of human lung diseases*
15:00-15:30	Invited lecturer: Evren Azeloglu (USA) - *Integrated multiomics for creation of a human kidney tissue atlas*
15:30-15:45	Selected oral presentation: Giorgia Egidy (France) - *Spatiotemporal analysis of melanoma heterogeneity*
15:45-16:15	Coffee break + Posters + Sponsors
16:15-16:45	Invited lecturer: Letizia De Chiara (Italy) - *Single Cell RNA sequencing to identify rare populations in com-*
16:45-17:00	Selected oral presentation: Lorenzo Giordani (France) - *Multimodal Spatial profiling of Neuromuscular Disorders*
17:00-17:30	Invited lecturer: Ying Zhu (USA) - *Deep spatial proteomics at cellular and subcellular resolution for human*
17:30-17:45	Selected oral presentation: Maria Elena Melica (Italy) - *Single-cell enable the identification of the molecular mechanisms driving drug-dependent restoration of the filtration barrier in crescentic glomerulonephritis*
20:00	Gala dinner in Florence (Baglioni Hotel)

Friday, June 30th 2023

Session 3 Circulating Biomarkers: Extracellular Vesicles & Single-Cells

Chairman: Michael W. Pfaffl & Patrice Martin

8:30-9:00	Keynote lecturer: Pieter Mestdagh (Belgium) - *Cell free RNA biomarkers in cancer*
9:00-9:30	Invited lecturer: Simon Carding (UK) - *Bacterial extracellular vesicle; Spheres of influence within and beyond the gut*
9:30-10:00	Invited lecturer: Michael W. Pfaffl (Germany) - *A development and analysis pipeline for microRNA biomarker signatures in molecular diagnostics based on circulating EVs*
10.00-10:15	Selected oral presentation: Matteo Cuccato (Italy) - *Extracellular vesicles miRNome profiling for sub-clinical mastitis detection*
10:15-10:45	Coffee break + Posters + Sponsors
10:45-11:00	Selected oral presentation: Davide Ferraro (Italy) - *Fast and reproducible method for extracellular vesicle isolation by novel droplet microfluidics device*
11:00-11:30	Invited lecturer: Christian Preusser (Germany) - *Using single-cell sequencing and Cre-lox reporters for tracking tumor EVs*
11:30-11:45	Selected oral presentation: Giulia Cantini (Italy) - *Circulating tumor cells: a novel technique for single cell isolation and analysis in adrenocortical carcinoma*
11:45-12:05	2nd Gold sponsor: QIAGEN, Monique van Eijndhoven & Cristina Gomez (The Netherlands) - *Single-nucleotide resolution sequencing (IsoSeek) of plasma EV-associated miRNAs for treatment response prediction in Multiple Myeloma patients*
12:05-14:00	Lunch + Posters + Sponsors: QIAGEN

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Session 4 Integrative Analysis - from multi-Omics to Bioinformatics

Chairman: Alexandra Whale & Daan Noordermeer

14:00-14:30	Keynote lecturer: Daan Noordermeer (France) - *Allele-specific multi-omics investigation of imprinted gene domains using phased long-reads*
14:30-15:00	Invited Lecturer: Benedikt Kirchner (Germany) - *Hidden layers of complexity - How small RNA distribution and sample handling affect sequencing results*
15:00-15:30	Invited lecturer: Chiara Lanzuolo (Italy) - *SAMMY-SEQ: A new technology to capture Dysfunctional Chromatin Landscapes*
15:30-15:45	Selected oral presentation: Adrien Dufour (France) - *Multiomics analysis of pig pre-implantation embryo*
15:45-16:15	Invited lecturer: Stephen Clark (UK) - *Single-cell multiomics sequencing to investigate the role of the epigenome in cell fate decisions during mouse embryogenesis*
16:15-16:30	Closing remarks

ORAL PRESENTATIONS

SESSION 1: NEW METHODOLOGICAL APPROACHES AND INSTRUMENTS IN MICROGENOMICS ANALYSIS

Spatiotemporal transcriptomic characterization of wound healing and regeneration in amphibians and of ischemic brain injury in mice

ZUCHA D[1,2], ABAFFY P[1], KIRDAJOVA D[3] , JIRAK D[4,5], ANDEROVA M[3], VALIHRACH L[1] , RADEK S[1], RAVINDRA N[1], KRAUS D[1], NETUSIL J[1], SMETANA K JR[6], LACINA L[6], BEDUYA EB[7], NEUZIL J[7], PSENICKA M[8], KUBISTA M[1]

Abstract

Using single cell profiling and spatial transcriptomics we characterize a new cell type critical for wound healing and in extreme cases regeneration in amphibian. The new cells are referred to as Regeneration Initiating Cells (RICs). The RICs are formed transiently from the basal epidermal cells and are critical for the modification of the surrounding extracellular matrix to allow for migration of other cell types such as regeneration organizing cells that further promote regeneration. Absence or deregulation of RICs leads to excessive extracellular matrix deposition and regeneration defects. We also characterize cellular processes that occur after ischemia in the mouse brain tissue during the first week of injury. The recovered spectrum of ischemia-induced oligodendrocyte states supports the emerging hypothesis that oligodendrocytes actively respond to and modulate the neuroinflammatory stimulus.

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Sketching chromatin dynamics at the single cell level

GIANNESE F, LAZAREVIC D, GIANSANTI V, DE PRETIS S, TONON G, CITTARO D[1]

Introduction

Single-cell ATAC-seq enables efficient profiling of epigenomes in heterogeneous cell populations, with unprecedented detail. Nevertheless, it queries only a minor fraction of the genome, as the vast majority is condensed in heterochromatin. We engineered a hybrid transposase including the chromodomain of protein HP-1α (TnH) and devised an ATAC-based strategy to study the accessible and compact chromatin in the same cells. This approach, named scGET-seq, expands the toolkit available to investigate cell phenotypes at the single cell level.

Material and Methods

A mixture of Tn5 and TnH transposases, each bearing a specific barcode, was used to tagment permeabilized nuclei. Nuclei isolation and barcoding is performed using 10x scATAC-seq protocol; alternative approaches, including sequential combinatorial barcoding, are possible. Transposase-specific reads were identified and processed separately. Read tags were assigned to fixed-size genomic bins to create two cell-by-bin count matrices, used to derive a single embedding. The probability of observing an excess of Tn5 signal in each window is used to model the local chromatin properties and identify cell states.

Results and Discussion

In multiple settings with cell mixtures (cell lines and PDOs), scGET-seq identified CNA at higher resolution compared to scATAC-seq, thanks to a more uniform genome coverage. Coupled with transcriptome data, it identified cell populations that could hardly be recognized otherwise. Analysis of continuous phenotypes, such as IPS differentiation, revealed the ability to describe chromatin (un)folding and identifiy epigenetic drivers. Lastly, scGET-seq recapitulated chromatin organization at a broad scale.

Conclusions

scGET-seq allows the concurrent profiling of accessible and compact chromatin in single cells extending the analysis of epigenomes to their dynamic context. In the future, scGET-seq can be adapted to most ATAC-based protocols, especially including multiomic approaches.

References

Tedesco M, Giannese F, et al., 2022. Chromatin Velocity reveals epigenetic dynamics by single-cell profiling of heterochromatin and euchromatin. *Nature Biotechnology* **40** 235-244.

Cittaro D, Lazarević D, Tonon G and Giannese F, 2023. Analyzing genomic and epigenetic profiles in single cells by hybrid transposase (scGET-seq). *STAR Protocols* **4(2)** 102176.

[1]Centero for Omics Sciences, IRCCS Ospedale San Raffaele, via Olgettina 58, 20132 Milano, Italy. cittaro.davide@hsr.it.

Doing more with less: comparative analysis of sample multiplexing methods for single-cell RNA-seq

MYLKA V[1].

Abstract.

Multiplexing of samples in single-cell RNA-seq studies allows a significant reduction of the experimental costs, straightforward identification of doublets, increased cell throughput, and reduction of sample-specific batch effects. Recently published multiplexing techniques using oligo-conjugated antibodies or -lipids allow barcoding sample-specific cells, a process called “hashing.” Here, we compared the hashing performance of TotalSeq-A and -C antibodies, custom synthesized lipids and MULTI-seq lipid hashes in four cell lines, both for single-cell RNA-seq and single-nucleus RNA-seq. We also compared TotalSeq-B antibodies with CellPlex reagents (10x Genomics) on human PBMC and TotalSeq-B with different lipid hashes on primary mouse tissues. Hashing efficiency was evaluated using the intrinsic genetic variation of the cell lines and mouse strains. Antibody hashing was further evaluated on clinical samples using PBMC from healthy and SARS-CoV-2 infected patients, where we demonstrate a more affordable approach for large single-cell sequencing clinical studies, while simultaneously reducing batch effects.

[1]Yvan Saeys, Data mining and Modelling for Biomedicine, VIB Center for Inflammation Research, Technologiepark-Zwijnaarde 71, 9052, Gent, Belgium.
Department of Applied Mathematics, Computer Science and Statistics, Ghent University, Ghent, Belgium.

Cellular specialization inside the symbiotic organ of an insect pest: deciphering the dialogue between host and symbiotic bacteria and their adaptation to different dietetic conditions

GALAMBOS N, BEVILACQUA C, VALLIER A, BALMAND S, GILLET B, HUGHES S, HEDDI A, PARISOT N AND ZAIDMAN-RÉMY A[1]

Introduction

Intracellular bacterial symbiosis (endosymbiosis) is widespread in nature and occurs particularly in insects living on nutritionally unbalanced diets. Cereal weevils (*Sitophilus spp.*) rely on the endosymbiont, *Sodalis pierantonius* that supplements their diet with metabolic components lacking, or scarcely available in cereal grains. *S. pierantonius* resides within specialized host cells, called the bacteriocytes, that group into the bacteriome organ. Such tissues function as hybrid prokaryotic-eukaryotic metabolic "factories", which exhibit a co-evolutionary genomic coadaptation between associated partners. Although, in a previous study, we showed that carbohydrate intake by the host triggers an exponential endosymbiont proliferation in young adults, little is known about the molecular dialog between the endosymbionts and the different insect cells that constitute the bacteriome organ, notably peripheral bacteriocytes, central bacteriocytes and epithelial cells (Figure 1). Thus, the aim of this study was to understand the connection between cell biology, transcriptomic regulation, nutrients.

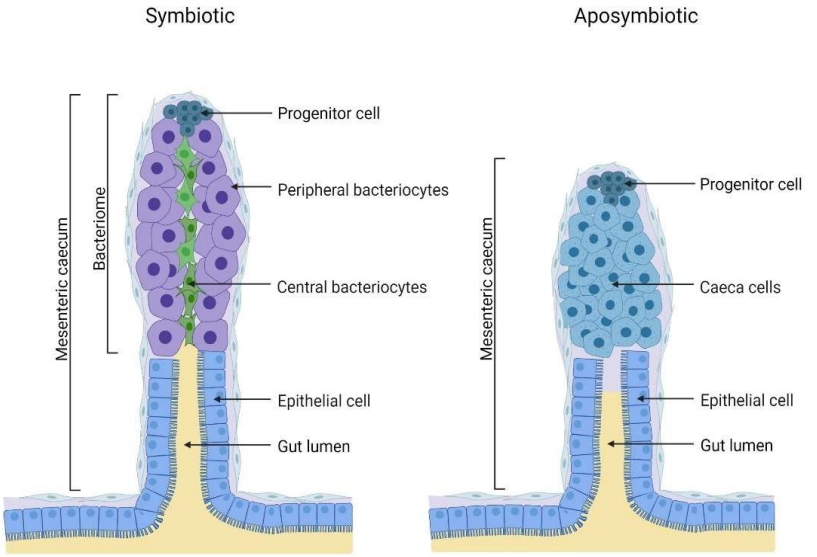

Figure 1. The different cell types that constitute the bacteriome organ in symbiotic metabolism and bacterial growth, and aposymbiotic weevils.

Material and Methods

First, to understand the cellular specialization inside the bacteriome, we applied low input dual RNA-sequencing on laser-capture micro-dissected bacteriocytes and epithelial cells from symbiotic insects and compared their transcriptomic profile with caeca and epithelial cells from insects artificially depleted of endosymbionts (aposymbiotics). To understand the metabolic dialogue between the different host cells and the endosymbionts under standard versus suboptimal nutritional conditions, we applied the same transcriptomic approach on laser-capture micro-dissected bacteriocytes and epithelial cells from insects fed on wheat grains, on a severely unbalanced diet (i.e. starch), or kept under starvation.

Results and Discussion

Here we describe an optimized microdissection protocol for low input dual RNA-sequencing to provide good concentration and high-quality RNA, from the different insect cells that constitute the bacteriome organ.

Conclusions

This work will help identify key pathways required for nutrient exchange inside different cell types forming the adult bacteriome, which plays a central role in fulfilling the important metabolic needs of the insect at different critical phases of insect growth, notably for the cuticle synthesis in emerging adults.

[1]Univ Lyon, INSA Lyon, INRAE, BF2I, UMR 203, F-69621, Villeurbanne, France. nikoletta.galambos@insa-lyon.fr anna.zaidman@insa-

Using digital PCR to characterise viral vector genomes

WHALE A[1]

Abstract

Viral vectors are an effective gene transfer method to modify a wide range of cells and tissues to produce therapeutic products to treat or prevent diseases. Before they can be introduced to the host, their safety and effectiveness must be determined by analysing critical quality attributes such as their identity, purity, potency, safety, and stability. Current analytical techniques are limited with low-throughput, large sample requirements, and poorly understood measurement variability. This presentation will focus on the development of a digital PCR (dPCR) method to characterise the genome of AAV particles (modified adeno-associated viruses). The focus is on identification and quantification of complete genomes (correct), partial genomes (incorrect) or empty (non-function) genomes within the capsid of the viral particle.

Briefly, dPCR sub-divides a quantitative PCR reaction by limiting dilution into thousands of sub-nanolitre reactions, termed partitions. PCR cycling is performed, each partition “read” and assigned as positive or negative based on the presence or absence of fluorescence, respectively. The copy number concentration is estimated using the proportion of positive partitions, with the application of Poisson statistics to account for partitions that initially contained more than one target. This approach means that a standard curve is not required to estimate the copy number concentration. Furthermore, multiplexing enables regions along the full length of the vector genome to be targeted and so enable quantification of full length genomes as those that are contained in a partition that is positive for all targets.

[1] Dr. Alexandra Whale, Science Leader, Molecular Biology, National Measurement Laboratory, United Kingdom.
Email: Alexandra.whale@lgcgroup

Examples of the duplex and multiplex ddPCR applications from the contract research organization practice

YAKUSHEV S[1]

Abstract

Microsynth provides support to biotech/pharma industry from R&D phase to the market authorization and routine release tests and acts as a reliable partner for the academy. To be successful and reliable partner the CRO should master instruments and methods, which are adequate for their intended use.

From the presentation you can learn detailed examples of the adequate and successful ddPCR application in Vector Copy Number quantification (VCN) and chromosomal translocation quantification in CAR-T cells, gene expression analysis in transgenic animals and quality and composition control of the food. Tips in mastering the ddPCR method and lessons learned from the CRO practice will sum up the talk.

[1]Microsynth AG, Schützenstrasse 15, 9436 Balgach, Switzerland. Email: sergey.yakushev@microsynth.ch

OPTO-Cas9 allows easy, fast and precise spatiotemporal genome editing in vertebrate embryo at cellular resolution

TISSERAND B, DÉBARE H, SCERBO P, ZHANG W, DELAGRANGE M, BENSIMON D AND DUCOS B[1]

Abstract

The discovery of CRISPR/Cas9 system has been a breakthrough for genome editing strategies. Although CRIPSR/Cas9 is mainly used for gene inactivation during embryonic development, the commonly used approaches still lack precise spatiotemporal control for gene inactivation, which occurs in the genome of all cells and tissues. Here we show that, by developing an optogenetic approach, we can quickly spatiotemporally modify gene activity by using a photo-controllable Cas9 fused to an estrogen receptor (ERT) domain (named OPTO-Cas9). As a proof of concept, we demonstrate that OPTO-Cas9 can be precisely targeted to inactivate a cell-type specific gene, tyrosinase (tyr) in pigmented cells of only one eye. Moreover, OPTO-Cas9 can allow precise analyses on embryonic morphogenesis by spatially knocking down the activity of pleiotropic genes, as we demonstrate for specific VENTX/NANOG zebrafish homolog (vox) gene inactivation in tailbud precursor cells. Together, OPTO-Cas9 enables quick photo-controllable local spatiotemporal inactivation of genes of interest in vivo at cellular resolution and we propose that OPTO-Cas9 can benefit other biological systems.

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Monitoring circulating tumor DNA during immunotherapy in Non-Small Cell Lung Cancer patients by targeted NGS: preliminary data on the CORELAB project

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Abstract

Liquid biopsy is a non-invasive promising strategy to detect and monitor circulating cancer derived material obtained from several body fluids instead of tumor tissue; it helps dissecting cancer heterogeneity and avoiding stressful and painful procedures. The analysis of circulating cell-free DNA (cfDNA) from plasma has given promising results in lung cancer. However, despite the increasing implementation of immunotherapy-based treatment, the prognosis of advanced NSCLC patients remains miserable, and therefore research of specific predictive biomarkers is actively pursued.

CORELAB is an ongoing multi-centric project sponsored by Regione Toscana (Italy), with the aim to discover new predictive biomarkers of activity and efficacy of immune check point inhibitors in NSCLC. Our group is specialized in liquid biopsy and contributes to the project analyzing the mutational status of plasma derived ctDNA.

From January 2021 to date, patients with NSCLC candidates for treatment with immune check point inhibitors have been consecutively enrolled in the CORELAB project. Blood samples were collected for each patient before immunotherapy (T0) and after 2, 4, 6 and 12 months (respectively T1, T2, T3 and T4) or until disease progression (TP). cfDNA was isolated from plasma, then quantified in terms of ng/µl using a QubitTM dsDNA HS Assay Kit and in the end the mutational status was evaluated by NGS sequencing using the OncomineTM lung cfDNA Assay, a panel of 11 lung cancer related genes (ALK, BRAF, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1 and TP53).

Here we present the preliminary data derived from the longitudinal analysis of liquid biopsy before and after treatment in advanced NSCLC patients, in particular referring to cfDNA quantification and mutational analysis by targeted NGS.

The adopted method reached high levels of sensitivity and specificity, detecting low allelic fraction mutations in cfDNA (in the range 0.1-0.5 % depending on the specific mutation). The most frequently mutated genes were TP53 and KRAS. The analysis of the results took into consideration the variations of ctDNA expressed both as VAF% and as nanograms of mutated DNA in particular referring to differences between T0 and T1. The majority of patients show a decrease in ctDNA after two months. We stratified the patients according to the quantity of ctDNA at baseline and after treatment and on the basis of the number of variants identified by NGS. The results were correlated with the clinical characteristics of the patients.

The longitudinal study of cfDNA allowed a dynamic monitoring of the disease through the assessment of the presence of specific tumor-related mutations and the evaluation of their rate over time. cfDNA was confirmed an easily accessible material for monitoring the mutational status of the tumor over time and could represent a useful prognostic or predictive biomarker in NSCLC patients treated with immunotherapy.

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dPCR vs. qRT-PCR for quantitative detection of SARS-CoV-2 RNA in environmental specimens

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Introduction

SARS-CoV-2 detection in wastewater samples proved to be an affordable method for large-scale screening, ensuring a continuous surveillance and a possible early-warning system (1). The aim of this study was the comparison of digital PCR (dPCR) and three quantitative Real Time PCR (qRT-PCR) assays for the quantification of SARS-CoV-2 RNA in wastewater samples.

Material and Methods

35 samples, collected from March-October 2021 at Ponte a Niccheri (Florence) wastewater treatment plant, were analyzed. After concentration and extraction, SARS-CoV-2 RNA was quantified using dPCR (One-Step RT-ddPCR Advanced Kit for Probes reagent kit, Bio-Rad Laboratories Inc., Hercules, U.S.A., using in-house primers and probe) and three qRT-PCR assays: in-house protocol with the same primers and probe of dPCR (2) [A]; Wastewater SARS-CoV-2 RT-qPCR System (Promega Corporation, Madison, U.S.A.) [B]; Quanty COVID-19v2 (Clonit Srl, Milano, Italy) [C].

Results and Discussion

SARS-CoV-2 RNA was detected by at least one method in 30/35 (85.7%) samples. SARS-CoV-2 RNA was detected in 19/35 (54.3%) samples with dPCR, and in 8/35 (22.9%), 14/35 (40.0%) and 19/35 (54.3%) with the A, B and C methods, respectively. Concordance was observed for five negative and three positive samples (22.9%). SARS-CoV-2 RNA mean copies/L was $2.5 \cdot 10^3$ for dPCR, compared to $3.0 \cdot 10^2$, $1.3 \cdot 10^4$ and $9.2 \cdot 10^3$ for the A, B and C methods, respectively.

Conclusions

dPCR achieved the same percentage of positive results of the commercial C assay, confirming a higher sensitivity (3) than the in-house method. The differences of SARS-CoV-2 RNA copies/L could be correlated with the different viral targets detected and with an additional freeze-thawing step occurred for dPCR. These results might suggest the need of more than one method to increase sensitivity.

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SESSION 2:
SOLID TISSUES AND SPATIAL OMICS

Omics at the bedside of human respiratory diseases

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The respiratory tract is an elaborate line of defense against outside aggressions that we describe in the Human Lung Cell Atlas (HLCA). The HLCA results from an integration of 49 independent datasets of the human respiratory system spanning over 2.4 million cells from 486 individuals. The HLCA defines consensus cell type markers and standards, and links gene expression to metadata about age, sex, body mass index, position along the proximal-to-distal axis of the bronchial tree. The resulting atlas can be used to annotate and interpret new lung diseases datasets. A large differential gene expression was found between identical cell types from nasal and tracheobronchial origins, in line with their distinct developmental origins, and their different exposures to noxious substances.

Next versions of HLCA will integrate additional multiomics information about RNA isoform expression, chromatin accessibility, cell surface protein expression, adaptive immune receptor repertoire profiling, DNA methylation, spatial information. We introduce ScNaUmi-seq to assess RNA isoform expression. ScNaUmi-seq combines Oxford Nanopore sequencing with an accurate cell barcode and UMI assignment strategy. We developed SiCeLoRe, a bioinformatics pipeline to explore the data, that works directly with long read sequencing data, but which can also integrate short read information. Application of long-read sequencing to spatial transcriptomics (Visium, 10X Genomics) resulted in SiT (Spatial isoform Transcriptomics), an approach to characterize spatial isoform variation and sequence heterogeneity on tissue sections. An online exploration resource was set-up (https://www.isomics.eu).

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From building a tissue atlas to actionable biomarker discovery, integrative multiomics in precision medicine

AZELOGLU, EU[1]

Introduction

Complementary unbiased assays, such as proteomics and single-cell transcriptomics can be integrated to decode the systems-level behavior of tissues; to gain mechanistic insights into physiological processes; and to identify prognostic or actionable biomarkers associated with disease outcomes.

Material and Methods

We show two use cases of integrative multiomics using pathway and network analytics: multiple transcriptomics assays, mass-spec proteomics, metabolomics, and CO-DEX imaging of healthy donor kidney biopsies and the unaffected segments of nephrectomies have been integrated to characterize human kidney tissue at the cellular level (Hansen *et al.* 2022). In addition, single-cell transcriptomics, isobaric urinary proteomics, and aptamer-based plasma proteomics combined with machine learning approaches are integrated to decode disease mechanisms in severe SARS-CoV-2 infection.

Results and Discussion

Single-cell transcriptomic assays, complemented with spatial or bulk proteomic methods, show that cell type-specific composition of the deep proteome can be spatially anchored within human kidney biopsies. Glomerular and tubular cells display different principal pathways that drive their physiological function and unique metabolic signatures that have potential implications in acute kidney injury. Post hoc power analysis shows that each cell type requires a unique sample size to determine a stable transcriptomic identity. Multiomic interrogation of biospecimens from patients hospitalized for severe COVID show unique urinary and plasma biomarker signatures that change with disease severity (Figure 1). A random forest algorithm, following Boruta feature selection, identifies urinary biomarkers that can successfully predict outcomes 5-13 days in advance. Multiomic networks projected onto kidney segment-specific genomes point to differing key disease processes specific to proximal tubules, podocytes and infiltrating immune cells.

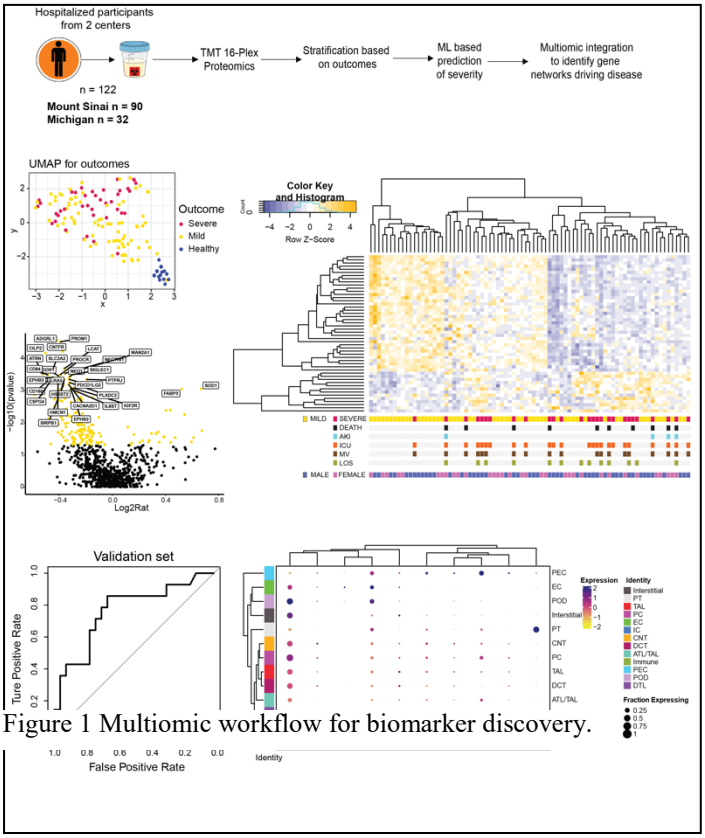

Figure 1 Multiomic workflow for biomarker discovery.

Conclusions

Cells, pathways, and segment-specific gene signatures can be used to recapitulate the human kidney tissue physiology and architecture using integrated multiomics. Integrated multiomics can also be used to decode kidney-specific disease mechanisms and affected cell types in severe COVID.

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Spatiotemporal analysis of melanoma heterogeneity in a spontaneous swine model at single-cell resolution

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Introduction

Melanoma is the deadliest skin cancer, with an increasing incidence especially in young adults. Melanoma may appear in nearly all pigmented animals. In the MeLiM minipig model, cutaneous melanomas develop during the perinatal period. They display many traits of malignancy as in humans. Intriguingly, MeLiM melanomas spontaneously regress with a concomitant immune response. In other words, this cancer is totally "cured" within 6 months, with no side effect other than lesion and coat depigmentation. Compared to current immunotherapies, this model provides cues for cancer treatment with a high success rate and few adverse effects. Single-cell RNA-seq and spatial transcriptomics analysis could give indications on intratumor heterogeneity at the individual level, investigating clonal evolution and the involvement of the microenvironment.

Material and Methods

Frozen samples spanning different time points during tumor evolution, from early lesions to progressive lesions sampled in couples of littermates were selected. Screening of conditions on nuclei isolation and purification for single-cell and depigmentation strategies for spatial analysis was achieved with RNA integrity and image analysis. Single-nucleus RNA-seq libraries and Visium transcriptomics were performed with the 10X Genomics technology.

Results and Discussion

We will present considerations for the best protocols adapted to our samples and a landscape of MeLiM lesions obtained after analysis of 61000 single cells. The analysis of cell types as cell cycle scores was consistent with immunofluorescence studies. Spatially resolved transcriptomics enabled to localize part of the cells identified in the single-cell libraries.

Conclusions

This study will help understand the regression mechanisms in MeLiM minipigs.

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Single Cell RNA sequencing to identify rare populations in complex organs – Relevance to the kidney

DE CHIARA L[1] AND ROMAGNANI P[1,2]

Introduction

The kidney is a complex organ composed of highly specialized cells, with limited capacity for proliferation and regeneration. Among the many insults that can harm the kidney, acute kidney injury (AKI) is one of the most frequent and it is characterized by a rapid deterioration in kidney function. AKI mostly affects the tubular compartment of the kidney driving tumorigenesis and triggering repair processes involving increased cell mitosis and polyploidization. Collectively, these responses lead to functional alterations at the cellular level. However, the processes that govern these responses are currently unknown. Here, we explore the utility of single cell RNA-sequencing (scRNA-seq) to identify the mechanisms driving kidney tubule adaptation to AKI.

Material and Methods

To identify specific injury and repair programs within the kidney tubule, we employed transgenic mouse models based on the FUCCI2aR and Confetti reporters. We further applied scRNA-seq analysis in vitro and in vivo after AKI combined with specific knocked-down experiments, cell sorting and pharmacological inhibition.

Results and Discussion

We found that following AKI the kidney responds via two main mechanisms: 1) polyploidzation-mediated hypertrophy of TC and 2) proliferation of renal progenitor cells (RPC). In vitro and in vivo scRNA-seq analyses at different days after AKI with fluorescent reporters revealed the activation of specific signatures in the two subsets of cells. Immediately after AKI, the expression of cell cycle markers identifies a population of DNA damaged polyploid TC. In vivo and in vitro scRNA-seq along with sorting of polyploid TC demonstrate that cells with DNA damage acquire a pro-fibrotic phenotype culminating in increased TGF-β1 expression. Interactome analysis revealed that TGF-β1 signaling fosters a reciprocal activation loop among polyploid TC, macrophages and fibroblasts. Conversely, RPC expand following AKI. Integrated analysis of publicly available datasets with a dataset generated from human renal progenitor cells identified a rare subpopulation as the origin of a subtype of kidney cancer.

Conclusions

In conclusion, we demonstrate that: 1) scRNA-seq can be successfully applied to characterize polyploid and progenitor cells if combined with the right tools and 2) scRNA-seq revealed the presence of a specific program in polyploid TC and renal progenitor cells.

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Multimodal Single Cell profiling of Duchenne Muscular Dystrophy

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Abstract

Duchenne muscular dystrophy (DMD) is one of the most severe pediatric degenerative myopathies. In the initial phase of the disease, muscle is exposed to continuous cycles of degeneration and regeneration; over time, regenerative potential is exhausted, and necrosis prevails. As of today, the cellular and molecular determinants responsible for this functional exhaustion remain largely uncharacterized.

Adult tissue repair requires the activation of resident stem cells that can both self-renew and generate differentiated progeny. To establish and maintain their properties, stem cells require constant interactions with their microenvironment and their neighboring cells that altogether constitute the niche. The stem cell and its niche form as a whole the minimum functional unit of adult tissue repair. Any given perturbation affecting either the stem cell or the molecular/cellular components of the niche will invariantly impact repair potential. Therefore, in DMD the changes hindering the correct execution of the repair process must therefore occur either in the stem cell or in its niche.

Here we present a multi-omic Spatial strategy to elucidate the determinants interfering with regeneration in the dystrophic muscle and study the niche-stem cell interactions. Taking advantage of snRNAseq, snATACseq and Spatial transcriptomics, during disease progression we profiled the evolution of muscle-resident cellular populations to identify dysfunctional subfractions and deregulated crosstalk.

Our data could serve as basis for future studies aimed at the identification of novel biomarkers and lay the foundation for new therapeutic approaches to promote muscle regeneration.

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Deep spatial proteomics at cellular and subcellular resolution for human and plant biology

ZHU Y[1]

Introduction

Mass spectrometry-based proteomics using bulk-scale and whole tissue profiling can provide exceptional measurement depth, but these approaches only capture averages that obscure single-cell or regional heterogeneity. With the advance of sensitive mass spectrometry, there is a growing interest in developing deep spatial proteomics technologies.

Results and Discussion

In this talk, I will give a brief introduction of our effort to integrate Laser-Capture Microdissection (LCM), nanodroplet Processing in One-pot for Trace Samples (nanoPOTS), and sensitive mass spectrometry methods. I will highlight the role of microfluidics in single-cell and spatial proteomics, as well as our effort to improve both spatial resolution and measurement throughput for spatial proteomics. Using the developed technologies in our lab, we can quantitatively profile >3500 proteins from a thin section of human pancreas tissue at a spatial resolution of 50 μm, corresponding to ~10 human cells. We also apply the platform to study the cell-type-specific proteome regulation of poplar leaf in response to environment stress such as drought. I will also share our current effort to improve the spatial resolution to subcellular scale using a laser ablation-based cell isolation method.

Conclusions

Our work demonstrates the coupling of LCM, nanoPOTS with mass spectrometry can become a key tool in spatial proteomics for both human and plant sciences.

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Single-cell enable the identification of the molecular mechanisms driving drug-dependent restoration of the filtration barrier in crescentic glomerulonephritis

MELICA M E[1], SEMERARO R [1], LASAGNI L[1] AND ROMAGNANI P[1,2]

Introduction

Recently we provided a mechanistic insight into crescent formation demonstrating that this lesion is caused by fast clonal proliferation of single immature renal progenitor cells (RPC) and by a lack of their differentiation toward a fully differentiated podocyte phenotype upon severe glomerular injury. We demonstrated that panobinostat, an HDAC inhibitor attenuate crescentic glomerulonephritis (CGN) by targeting the causative uncontrolled hyperplasia of immature RPC into a controlled differentiation into new podocytes and thereby restoring the injured glomerular filtration barrier. Understanding the beneficial effect of panobinostat could accelerate the translation of these results into clinical practice, thus we aimed to analyze the effect of panobinostat on the pathways involved in the RPC-to-podocyte differentiation.

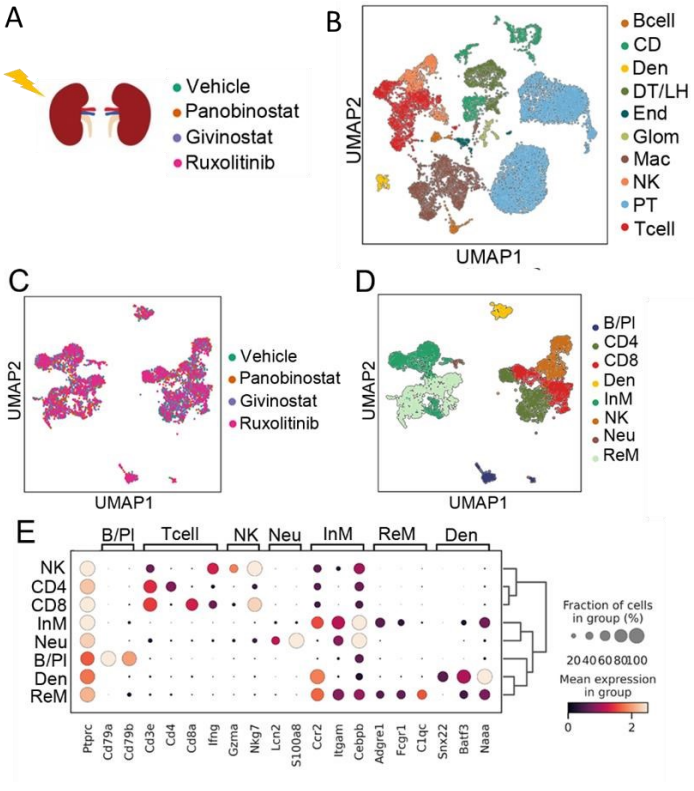
Material and Methods

We established a CGN model in a conditional transgenic mouse that allows lineage tracing of RPC. Single cell RNA sequencing (scRNAseq) of kidney of mice with CGN treated with vehicle or panobinostat highlighted the major pathway driven CGN amelioration. Human RPC cultures were treated with panobinostat or with inhibitors of the putative pathway, we assessed differentiation by gene expression, then verified at protein level by super-resolution microscopy.

Results and Discussion

To evaluate the mechanisms by which panobinostat drives the differentiation of

RPC into podocyte we performed scRNAseq on kidney of mice with CGN treated with vehicle or panobinostat and we evaluated the most modulated genes. We focused on the pathways already known for their role in RPC-to-podocyte differentiation and we identified a series of genes specifically regulated by panobinostat. To corroborate our results, we performed similar analysis in a scRNAseq dataset of murine progenitors isolated from mouse kidney progenitors treated in vitro with panobinostat. The pathways emerging from bioinformatics analysis were validated by *in vitro* experiments on human RPC.



Conclusions

Our results clearly identify the molecular mechanisms by which panobinostat exert its unique capacity of targeting the uncontrolled hyperplasia of immature RPC into a controlled differentiation into new podocytes which ultimately reverse crescent formation and improves clinical outcome.

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SESSION 3:
CIRCULATING BIOMARKERS:
EXTRACELLULAR VESICLES & SINGLE-CELLS

Patient-specific alterations in blood plasma cfRNA profiles enable accurate classification of cancer patients and controls

MESTDAGH P[1]

Circulating nucleic acids in blood plasma form an attractive resource to study human health and disease, including cancer. To date, most studies on circulating nucleic acids in cancer patients have focused on circulating tumor DNA. Cell-free RNA (cfRNA) may complement ctDNA by reflecting dynamic changes in gene expression during cancer development and progression, or upon treatment, in both healthy and diseased cells. We applied mRNA capture sequencing of blood plasma cell-free RNA from 266 cancer patients and cancer-free controls. We observed cancer-type specific as well as pan-cancer alterations in cell-free transcriptomes compared to controls. Differentially abundant RNA were heterogenous among patients and among cohorts, hampering identification of robust cancer biomarkers. Therefore, we developed a novel method that compares each individual cancer patient to a reference control population to identify so-called biomarker tail genes. These biomarker tail genes discriminate ovarian, prostate, and uterine cancer patients from controls with very high accuracy (AUC = 0.980). Our results were confirmed in additional cohorts of 65 plasma donors (2 lymphoma types) and 24 urine donors (bladder cancer). Together, our findings demonstrate heterogeneity in cell-free RNA alterations among cancer patients and propose that case-specific alterations can be exploited for classification purposes.

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Bacterial extracellular vesicles: spheres of influence within and beyond the gut

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Introduction

The human gastrointestinal tract is home to hundreds of trillions of microorganisms (the microbiome) that perform a vital role in food digestion and providing essential nutrients and vitamins. They also play an important role in metabolizing medicines and drugs, and in resisting infection by pathogens. Gut microbes are, however, susceptible to change with alterations in their makeup and activity occurring as a result of exposure to various environmental factors such as diet, drugs, pathogens and behaviour. Such changes have been associated with more than 90% of human diseases affecting the gut, liver, joints, heart and brain. A central question to discriminating between association and causality is, how do gut microbes communicate with their host to affect physiological changes at the cellular and organ level within the gut and beyond? We have uncovered roles for microbiota-derived metabolites and highly stable, nanosized microvesicles (bacterial extracellular vesicles; BEV) naturally produced in the gut by prominent members of the intestinal microbiota in cross-kingdom communication (1).

Material and Methods

BEV produced by the human gut commensal bacterium Bacteroides thetaiotaomicron (VPI-5482) are isolated from cultures using chemically defined media and by sequential filtration, ultracentrifugation and size exclusion chromatography (2). Biophysical, biochemical and immunological assays are used to characterise BEV and ensure consistency prior to downstream applications. Various in vitro single and multi-cell, -tissue and -organ culture systems are used in combination with pre-clinical in vivo models to assess the biological properties of BEV and evaluate the therapeutic potential of BEV engineered to deliver biologics and vaccines.

Results and Discussion

We have shown that specific bacteria and their metabolites can affect various sensory cells of the immune, endocrine and nervous systems within the intestinal mucosa and that BEV can bring about changes in host cell physiology by delivering various cargo including metabolic enzymes and mediators of intracellular signaling. Via their ability to cross the intestinal epithelium and access the lymphatic and vascular system they can also affect cells and tissues throughout the body and can interact with key cells of the immune system to promote local and systemic regulatory responses. Furthermore, we have exploited this BEV-mediated cross-kingdom communication pathway to develop a drug delivery technology platform using BEV to deliver therapeutic proteins and vaccine antigens directly to mucosal tissues. Pre-clinical studies highlight the utility of this technology in both boosting natural immunity and in preventing and treating infection and autoimmune mediated pathologies that affect the gut and other tissues.

Conclusions

Our findings to date provide new insights into the nature of host-microbe interactions in the gut and the role that BEV play in maintaining microbial and immune homeostasis. They also provide the basis for investigating the potential of Bt BEV as an immune therapy.

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A development and analysis pipeline for microRNA biomarker signatures in molecular diagnostics based on circulating EV

PFAFFL MW[1]

Introduction

Extracellular vesicles (EVs) circulate in body liquids and are involved in intercellular communication and have significant regulative functions in almost any physiological or pathological process. Especially exosome-like small EVs have gained huge scientific and clinical interests because of their specific RNA biomarker signature, which is of high value in clinical and molecular diagnostics. Herein we focus on the methodological and bioinformatic challenges to create a valid microRNA biomarker signature.

Material and Methods

The past decade has brought about the development and commercialization of a multitude of extraction methods to isolate and purify EVs, primarily from blood liquid biopsies. The EV purity, concentration and which subpopulations are captured strongly depend on the applied EV isolation and analysis method. This determines how suitable resulting EV samples are for potential downstream transcriptomic applications, in small-RNA sequencing, quantitative RNA measurements, e.g. by RT-qPCR, for microRNA biomarker discovery. Hence, an overall transcriptomics analysis pipeline was developed, optimized and validated on various clinical patient cohorts. To fully analyze the small-RNA deep sequencing results various self-developed bioinformatical tools were used: (1) microRNA analysis pipeline (caRNAge), (2) analysis of microRNA isoforms (isomiRROR), and (3) identification of stable microRNA references (miREV). Differential expressed microRNA candidates were identified by multivariate statistics (e.g. HCA, PCA, PLS-DA) to find a reliable and stable biomarker signature.

Results and Discussion

Applied isolation and characterization methods were benchmarked with focus on their suitability for microRNA biomarker discovery as well as biological characteristics of captured EVs, according to the latest MISEV 2018 guideline. Various independent patient cohorts were investigated: healthy volunteers, mild- or severe pneumonia, acute pulmonary failure (ARDS), and patients with septic shock. Distinct microRNA signatures could be identified, which are applicable to indicate disease progression from healthy, over limited inflammation present in pneumonia, to severe inflammatory changes in ARDS, or septic shock.

Conclusions

These study results indicate that EV microRNA biomarkers have a high potential for early diagnosis in lung inflammation events. Furthermore, the methodological findings provide guidance for navigating the multitude of EV isolation methods available, and help researchers and clinicians in the field of molecular diagnostics to make the right choice about the optimal isolation strategy and bioinformatical analysis of huge transcriptomics datasets to get valid EV associated biomarker signatures.

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Extracellular vesicles miRNome profiling for subclinical mastitis detection

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Introduction

Subclinical mastitis represents a relevant issue in dairy herds for clinical sign absence, reducing the possibility of early treatments. Diagnosis is done by detecting an increase in somatic cell count (SCC). Intriguingly, miRNA vehiculated by extracellular vesicles (EV) seem to have a remarkable role in the regulation of bovine mastitis [1]. The main aim of this study was to investigate the milk EV miRNome during subclinical mastitis.

Material and Methods

Milk samples were collected from 174 dairy cows. EV were isolated by size exclusion chromatography (SEC) columns and characterized by western blot analysis using TSG101 and CD9 markers. miRNA extraction was performed using an automated extractor and small RNA-sequencing was conducted on selected samples. The differential analysis was conducted using an SCC value of 200,000 cells/mL to allocate samples in each group. Functional analysis to predict miRNA role was performed using miRWalk, OmicsNet and Cytoscape.

Results and Discussion

Among 1997 differentially expressed microRNA, a total of 68 miRNA were identified with FDR value < 0.05. The main downregulated miRNA (miR-455-5p, miR-1301-3p and miR-503-5p) regulate many biological processes, such as innate immunity and inflammation. miR-1301-3p has been reported in cows experimentally infected with *Staphylococcus aureus* [2], but no studies in spontaneous bovine mastitis have been published. miR-455-5p has an anti-inflammatory activity in humans and its downregulation in our samples could be related to the onset of subclinical mastitis [3].

Conclusions

In conclusion, investigating the miRNA role in subclinical mastitis could allow a better understanding of the disease and an improvement in its diagnosis and management.

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Fast and reproducible method for extracellular vesicle isolation by novel droplet microfluidics device.

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Introduction

Extracellular vesicles (EV) represent a promising biomarker for different diseases, including various cancer types, cardiovascular and neurodegenerative diseases [1,2]. However, EV investigation requires efficient isolation methods, and this task is not satisfied by conventional techniques. Microfluidics, which allows handling small volumes in microchannels, can offer favorable strategies to face this aspect [2,3]. Here, a new droplet microfluidic device based on immunocapturing is presented and validated for EV isolation.

Material and Methods

Droplets encapsulating EV and magnetic beads are generated by the microfluidic device for the isolation protocol. Breast cancer cell line (MDA-MB-231) is used as EV source. Purified sample is characterized by: Nanoparticle Tracking Analysis (NTA) and Flow-cytometry (FC) for EV quantification, ii) BCA and western blot (WB) for protein characterization, iii) confocal microscopy for EV visualization, and iv) qRT-PCR for miRNA cargo analysis.

Results and Discussion

The novel and automated droplet microfluidic device allows for a good extraction rate (56%) (Figure 1a) within a short isolation time (2 mL in 4 hours); then, WB (figure 1b) and confocal imaging prove the presence of EV in the output sample, and miRNA analysis confirms their genomic content.

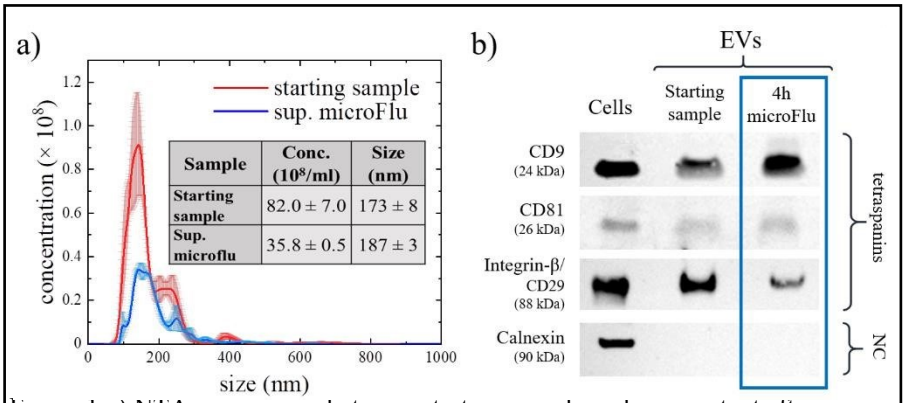

Figure 1 a) NTA comparison between starting sample and supernatant after microfluidic purification; b) WB showing the presence of common tetraspanins used for EV characterization; Calnexin was used as negative control.

Conclusions

EV purification assays performed by the droplet microfluidic device results in agreement with other isolation methods, but leading to faster isolation and higher processability volumes. Purification from more complex samples are currently under investigation.

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Using single-cell sequencing and Cre-Lox reporters for tracking tumor EV

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Introduction

Pancreatic cancer (PAC) presents a major challenge due to its elusive symptoms and resulting in late detection and limited treatment options. Extracellular vesicles (EVs) released by pancreatic cancer cells can impact the tumor microenvironment (TME) by transferring their cargo to recipient cells. Here, the Bcl2-associated anthogen 6 (BAG6) plays a critical role in the specific formation of the EV signature and its related impact on tumor progression. To investigate the effects of EV on specific cells in vivo, we used a Cre-loxP reporter mouse model for PC.

Material and Methods

Using Bag6-positive and Bag6-deficient tumor cells, we investigated the impact of Bag6 on tumor growth and aggressiveness in a preclinical mouse model of PC. EV released by tumor cells containing cre mRNA were transplanted into floxed dTomato reporter mice to track EV uptake at the individual cell level by using immunofluorescence microscopy and single-cell RNA sequencing.

Results and Discussion

Pancreatic cancer (PAC) presents a major challenge due to its elusive symptoms and resulting in late detection and limited treatment options. Extracellular vesicles (EV) released by pancreatic cancer cells can impact the tumor microenvironment (TME) by transferring their cargo to recipient cells. Here, the Bcl2-associated anthogen 6 (BAG6) plays a critical role in the specific formation of the EV signature. Of note, uptake of EV within the tumor microenvironment induced significant changes with respect to host cell infiltration and the phenotype of tumor infiltrating cells.

Conclusions

Using an integrated Cre-loxP approach in combination with single-cell sequencing, we were able to track the in vivo transfer of tumor EV and identify the recipient cells involved. This greatly improves our understanding of EV to shape the phenotypic and functional features in PC.

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Circulating tumor cells: a novel technique for single cell isolation and analysis in adrenocortical carcinoma

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Introduction

Adrenocortical carcinoma (ACC) is a rare aggressive tumor with poor prognosis when metastatic. Radical surgery still represents the best option for ACC treatment. Circulating tumor cells (CTCs) have been detected in ACC patients and their number seems prognostic. The non-standardizable detection approach with cell filters used so far in ACC for CTC isolation can affect the clinical value of this marker and limits genetic variance analysis at single-cell level. In this study, we aimed to develop a new and more specific method for single CTC isolation in ACC patients.

Methods & Results

Blood samples collected in Streck tubes from 5 ACC patients were subjected to size-based enrichment with the Parsortix Technology (Angle PLC, UK). Recovered cells were fixed, permeabilized immunostained for CD45 and with antibodies against the Steroidogenic factor 1 a nuclear marker of steroidogenic cells, for leucocytes (WBC) and adrenocortical CTCs, respectively. The immunostained cells were imaged and sorted with the DEPArray system (Silicon Biosystems). Image-based selection was used to identify and recover individual cells as either single cells or pools of cells, based on their morphological features, DNA content, and fluorescence labeling, namely CTCs (SF1-PE+/CD45-APC−/DAPI+) and WBC (SF1-PE−/CD45-APC+/DAPI+). A low pass whole genome sequencing was performed. Whole genome amplification was applied on DNA from the selected single-cells using the Ampli1 WGA kit version 02 (Silicon Biosystems) to explore CTC genomic heterogeneity and compare the genetic profiles between CTCs and leucocytes isolated from the same sample.

Conclusions

Our study allowed the set-up of a standardized method to isolate single CTCs in ACC patients. We applied for the first time to ACC a combination of Parsortix/DEParray technologies with a specific nuclear immunostaining to isolate CTCs and demonstrate their adrenocortical origin in order to evaluate their mutational profile at single cell level. This new procedure allows the genetic analysis of individual CTCs for the detection of chromosomal alterations/copy number variants. This approach will be useful to drive personalized post-surgery therapies in ACC patients.

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Single-nucleotide resolution sequencing (IsoSeek) of plasma EV-associated miRNA for treatment response prediction in Multiple Myeloma patients.

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Abstract

Recent advances show that apart from 21nt mature miRNA, miRNA with non-templated additions (NTA), called isomiR, have biological relevance that can dramatically change their targetome and add an extra layer of information to circulating miRNA biomarkers. Deconvolution of these posttranscriptional modifications is challenging in particular for low-input samples, e.g. plasma extracellular vesicles (pEV) that are considered as a source for minimally-invasive diagnostics. Both for biological studies as well as for diagnostic purposes accurate detection and distinction of mature miRNA and isomiR are essential. RNA sequencing is currently the only reliable method that can distinguish mature miRNA from isomiR with single-nucleotide resolution and that can truthfully reveal isomiR diversity in biological samples. However, current small RNA sequencing strategies remain imprecise, and sequencing artifacts identified as artificial isomiR might bias biological inference and therefore need to be ideally avoided. We developed “IsoSeek” that diverges from these methods by making use of randomized 5’- and 3’-adapters combined with a 10N unique molecular identifier (UMI). Using synthetic mature miRNA and isomiR spike-ins, we show that IsoSeek has reduced ligation and PCR amplification bias leading to improved accuracy in the detection of isomiR. When sequencing pEV samples we show that IsoSeek captures the full complexity of isomiR in comparison with commercial protocols. Moreover, we made a comprehensive evaluation of 10 different small-RNA sequencing protocols, showing that randomized-end adapter protocols showed superior accuracy for isomiR profiling. Our results highlight the relevance of protocol choice for biological isomiR detection and annotation.

Using machine learning on IsoSeek data from 43 healthy donor and 34 Multiple Myeloma (MM) patient pEV, we generated a signature that is able to discriminate MM patients with active disease from healthy controls (AUC=0.94; CI:0.88-0.97). Moreover, the addition of isomiR as independent features enhances discrimination of MM patients with active disease (n=34) from clinical responders (n=37) (AUC=0.94; CI:0.81-0.99).

IsoSeek advances our knowledge of miRNA diversity and provides proof of principle for using EV-associated miRNA sequencing data as a liquid biopsy method, e.g. to predict response to treatment.

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SESSION 4:
INTEGRATIVE ANALYSIS -
FROM MULTI-OMICS TO BIOINFORMATICS

Molecular diagnostics of allele-specific DNA methylation status at imprinted domains in mouse and human cells using Nanopore sequencing

NOORDERMEER D[1]

Introduction

Genomic imprinting is the epigenetic process in mammals, whereby several hundreds of genes are mono-allelically expressed depending of their parental origin (Ferguson-Smith, 2011). About half of all imprinted genes are clustered within about 20 genomic domains, where their mono-allelic activity is coordinated by specialized regulatory regions, so called Imprinting Control Regions (ICRs). ICRs carry mono-allelic DNA methylation marks (in a CpG context) and therefore constitute a subset of Differentially Methylated Regions (DMRs). Genomic imprinting has relevance for human disease and for animal breeding (Duranthon and Chavatte-Palmer, 2018; Monk et al., 2019). Imprinting disorders in human patients cause growth defects and mental dysfunction, like for instance in the Beckwith-Wiedemann and Angelman syndromes. DNA methylation changes at imprinted domains reduce developmental potential, thereby impacting the efficiency of Assisted Reproductive Technologies for both human and agricultural applications.

Molecular diagnostics of DNA methylation status at imprinted domains has traditionally been a laborious and complicated procedure, which requires a variation-prone chemical conversion process, creates small DNA fragments (100-200 bp) and has limited capacity to distinguish the parental alleles. Here I will present our ongoing progress to use Nanopore sequencing to determine parental origin and DNA methylation from long (multi-kilobase) native DNA molecules (Simpson et al., 2017).

Results and Discussion

To validate our approach, we applied Nanopore sequencing on mouse embryonic stem cells originating from known F1 hybrid crosses. Using a dedicated targeting strategy, we obtained up to several hundreds of long Nanopore reads (10 – 100 kb) that cover multiple imprinted domains in a single experiment. Using SNPs, we unambiguously assigned these native DNA reads to their maternal or paternal origin, followed by the identification of methylation status at individual CpGs. These results confirmed that DNA methylation status of CpGs at imprinted DMRs was mostly congruent in individual cells. Yet, they also identified interspersed regions that are bi-allelically hyper- or hypomethylated. This combined approach to call parental origin and DNA methylation of native DNA reads therefore provides a promising strategy for molecular diagnostics of domain-wide imprinting status. In collaboration with clinicians, we are currently applying this approach to *in-vitro* differentiated cells obtained from human imprinting disorder patients, where we aim to simultaneously phase the parental chromosomes and their methylation status.

Conclusions

The capacity of Nanopore sequencing to simultaneously determine parental origin and DNA methylation status provides a promising strategy for the molecular diagnostics of imprinting perturbations in human disease and for animal breeding.

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Hidden layers of complexity - How small RNA distribution and sample handling affect sequencing results

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Introduction

In recent years, small RNA species have emerged as prominent subjects of transcriptomic investigations, primarily owing to their substantial potential as prognostic and diagnostic biomarkers, as well as their pertinence to a wide range of physiological and pathophysiological pathways. Simultaneously, advancements in Next-Generation sequencing technologies have unveiled an unforeseen intricacy of small RNA transcripts within tissues and biofluids, particularly in liquid biopsies. This heterogeneity arises not only from diverse RNA species, including miRNA, tRNA, snRNA, snoRNA, and others, but also encompasses functionally active isoforms and fragments, such as isomiRs and tRFs. Furthermore, accurate analyses are complicated by the presence of batch effects introduced by isolation methods or sample handling, as well as the accurate identification of stable reference genes, which significantly impact the success of downstream validation through independent methodologies. Regrettably, the wealth of information made accessible by current sequencing technologies is often underutilized due to the intricate nature of transcripts and the associated challenges in bioinformatics.

Results and Discussion

Differential gene expression is directly dependent on relative abundances of the small RNA species in question. Amplified by the size selection bias inherent in most library preparation protocols, the presence of degradational products or over-represented functional RNA such as tRNA or yRNA can drastically alter normalization and therefore downstream statistics. In addition, analyses on gene level basis might distort important differences in expression for isoforms of small RNA transcripts such as isomiRs. To ensure accurate detection free from such biases, comprehensive analysis of the complete spectrum of small RNA as well as extensive quality control is of utmost importance.

Conclusions

We have developed a user-friendly computational pipeline that integrates established tools and custom reporting scripts to accommodate both experienced and novice analysts. This pipeline encompasses essential stages, such as quality control with batch effect detection, trimming, alignment considering transcriptional isoforms and functional fragments, differential gene/transcript expression, unsupervised and supervised clustering, as well as pathway analysis to identify overrepresented targets. With its comprehensive outputs in explorative html files, our pipeline, known as caRNAge, aims to address a wide range of scientific questions. Researchers can freely access the pipeline at https://www.physio.wzw.tum.de/caRNAge/.

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SAMMY-seq: A new technology to capture dysfunctional chromatin landscapes

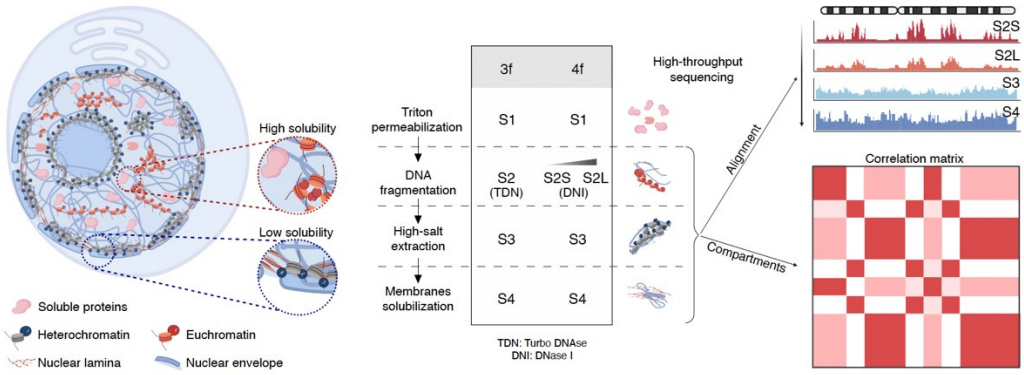
LANZUOLO C[1]

Introduction

The correct 3D organization of the genome is known to influence the spatiotemporal expression of lineage-specific genes during stem cell differentiation and aging processes. Alterations of chromatin architecture have been described in cancer or other pathologies.

Material and Methods

We introduce the SAMMY-seq technique to precisely map genomic regions separated by their biochemical properties. This single-handed technique enables the identification of heterochromatic and euchromatic domains and their compartmentalization in the nuclear space. Crucial practical advantages of this method include: its applicability on as little as 10K cells; reduced costs; few manipulation steps and short execution time.



Results and Discussion

We applied SAMMY-seq on fresh biopsy specimens from prostate carcinoma, a tumor type characterized by high heterogeneity and multifocality. In bulk healthy tissues, SAMMY-seq allowed the detection of a conserved pattern of euchromatin/heterochromatin domains associated with the respective epigenetic signatures. On the contrary, biopsies from cancer patients are characterized by different degrees of epigenetic aberrations, reflecting the heterogeneous nature of prostate tumours.

Conclusions

Our data highlight the impact of chromatin architecture on dysfunctional genome usage and propose SAMMY-seq as an eligible tool to shed light on chromatin solubility and epigenetic remodulations.

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Multiomics analysis of pig pre-implantation embryo

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Introduction

The embryonic development of the pig differs from that of humans and mice from the blastocyst stage and is characterized by a much later implantation. This particular period is concomitant with a lengthening and a significant growth of the extraembryonic tissues of the embryo and is still poorly understood. These drastic changes occurring before implantation could thus affect the biology of embryonic pluripotent cells in a new way compared to our knowledge developed on primate or rodent cells [1], [2].

Material and Methods

To better understand the biology of the pig blastocyst, we generated a large dataset of single-cell transcriptomics (scRNAseq) and multi-omics (paired RNAseq and scATAC-seq) at different embryonic stages (early, late, ovoid and elongated blastocysts) and the associated proteomic dataset from the corresponding uterine fluids. The analysis of this large dataset allowed us to identify the different cell lineage and to proceed to their functional characterization. We then inferred gene regulatory networks working on modules of gene regulation (regulon) [3] and we connected those regulons to ligand receptor interactions and to proteins detected in the uterine fluids. The single-cell multi-omics dataset (paired scRNAseq and scATACseq) helped us to validate gene networks and modules of gene regulation at work in the different cell populations and to characterize chromatin specific landscapes between the three main populations.

Results and Discussion

Our results confirm the molecular specificity of the three primary embryonic lineages (epiblast, trophectoderm and hypoblast) and identify stage-specific subpopulations. This allows us to infer the biological functions of these three main lineages and the interactions between them and identify key regulation modules linked to those functions. We also provide new insight into the biology of epiblast cells prior to implantation, and we observed potential interaction between the uterine fluids and the receptor inferred from the embryos.

Conclusions

This work provides new potential candidates for regulatory pathways controlling the development of pig embryos and provides new key transcription factors controlling pluripotency in the pig embryos. These results will allow the identification of new cytokines and inhibitors to improve the stability and derivation of porcine pluripotent cell lines from the epiblast and trophectoderm from porcine embryos.

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Single-cell multiomics sequencing to investigate the role of the epigenome in cell fate decisions during mouse embryogenesis.

CLARK S[1].

Abstract

Single-cell sequencing technologies are transforming our understanding of the gene expression dynamics of developmental biology, yet the role of the epigenome is less well understood. We recently developed a technique, named scNMT-seq for profiling DNA methylation, chromatin accessibility and gene expression from the same single-cells. Applying this method to cells collected from early mouse embryos we discovered an asymmetry in the formation of the three primary germ layers with the mesendoderm epigenome arising upon differentiation but the ectoderm profile being pre-specified in the early epiblast. Using genetic perturbations we next revealed a role of active TET-dependent demethylation in the enhancer landscape of early organogenesis which is necessary for the formation of the primitive blood lineage. Finally we developed a computational tool to predict transcription factor binding events and infer gene regulatory networks. We show that these can be used to generate in silico predictions of the effect of transcription factor perturbations which we validate experimentally by showing that Brachyury is essential for the differentiation of neuromesodermal progenitors to somitic mesoderm by priming cis-regulatory elements. In conclusion, single-cell multi-omics are powerful technologies for investigating the role of the epigenome in cell fate decision making.

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POSTER PRESENTATIONS

P1- Exosome micro-RNA as liquid biopsy biomarkers for skin melanomas

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Introduction

In the last decade, cutaneous melanoma incidence has dramatically increased and early diagnosis is crucial for appropriate tumor management. Liquid biopsy has attracted attention as a non-invasive method for early diagnosis, monitoring and treatment response evaluation. There is an urgent need for new biomarkers to follow up patients with melanoma negative for BRAF and/or NRAS hot spot mutations [1].

Material and Method

In this prospective multicenter study, blood samples for miRNA profiling were obtained from consecutive patients with a clinical and dermoscopic suspected diagnosis of melanoma and a control group without melanoma. Seven miRNAs, namely hsa-miR-149-3p, hsa-miR-150-5p, hsa-miR-21-5p, hsa-miR-200c-3p, hsa-miR-134-5p, hsa-miR-144-3p and hsa-miR-221-3p were profiled by quantitative PCR (qPCR) in plasma from patients with malignant melanoma (MM) and age and gender-matched controls.

Results and Discussion

Our results showed that three out of seven miRNAs, namely hsa-miR-200c-3p, hsa-miR-144-3p and hsa-miR-221-3p were differentially expressed in plasma-derived exosomes from melanoma patients and controls. Furthermore, the abovementioned miRNAs varied significantly with melanoma stage (I-II vs III-IV). The three biomarkers combined were able to discriminate by ROC curve analysis nevi from melanomas (Figure 1).

Conclusion

The combined expression level of hsa-miR-200c-3p, hsamiR-144-3p and hsa-miR-221-3p resulted to be a strong candidate biomarker for discriminating between nevi and melanoma with high accuracy. If validated, this finding has possible implications both for early diagnosis and follow-up procedures.

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P2 - Measles Di Quantification by Droplet Ditial PCR

ROUSSEAU C, MURA M, DUPONT E, VIGNE C, BARBIER L[1]

Introduction

Defective interfering (DI) genomes are truncated viral genomes generated during viral replication. DI genomes compete with the full-length viral genome replication, leading to their propagation. In line with vaccine immunogenicity studies, there is a need for the development of tools that detect and quantify DI genomes. Measles, one of the most contagious human diseases, is caused by the measles virus, a single-stranded RNA virus belonging to the order of *Paramyxoviridae*. Measles virus produce copy-back DI genomes leading to interferon-1 secretion by infected cells (Mura et al., 2017). For DI's relative quantification, RT-PCR can be used but requires standard curve analysis (Mura et al., 2017). In our institute, we sought to develop a new protocol of DI quantification using droplet digital PCR (ddPCR). This technology is a highly sensitive and precise method that offers an absolute quantification without the need of standard curves. This method is also less susceptible than RT-PCR to inefficient amplification caused by primer mismatch that can be strongly observed with DI genomes.

Material and methods

In order to quantify DI genomes on RNA extracts from measle-infected cells, reverse transcription followed by a ddPCR protocol (RT-ddPCR) was performed on the Bio-Rad QX200 plateform. First, several optimizations regarding annealing temperature, primers and probes concentrations were conducted. Then we used a ddPCR multiplex study using different sets of primers labeled with different fluorophores to quantify whole genomes and DI genomes on samples presenting a very low input of virus. Both results and data of ddPCR were generated and analyzed by quantasoft analysis system (Bio-Rad).

Results and Conclusion

RT-ddPCR provides accurate and sensitive identification and quantification of various models. Optimizations for improved quantification of measle DI genomes in our model of infected cells are in progress. The validation of such a ddPCR protocol will offer the ability to detect and quantify DI genomes in measle virus models and improve our knowledge of DI's consequences on immunogenicity. This technical development could be useful to quantify DI in other replicative virus models.

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P3 - New predictive biomarkers of efficacy of immune check point inhibitors in advanced NSCLC

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Introduction

In patients with advanced non-small cell lung carcinoma (NSCLC), immune checkpoint inhibitor and chemotherapy represent two distinct first-line standard-of-care regimens without clear and established biomarkers to inform the optimal choice for individual patients. PD-L1 expression, is approved as a predictive factor for efficacy of immune check point inhibitors in NSCLC. However, PD-L1 evaluation has had some limitations. Level of Tumor Mutation Burden has been proposed as new predictive factors of response in immunotherapy. However, it is still unknown whether TMB can be a useful biomarker. The therapeutic changes in advanced NSCLC have coincided with the increasing use of minimally invasive methods of tissue sampling and, consequently, with the reduction in sample size, which has important implications for pathological diagnosis.

Material and Methods

Fifty-three patients diagnosed with advanced NSCLC, without history of previous or concomitant other neoplastic diseases, candidates to treatment with ICIs as first or second line of treatment have been accrued by UOC Oncology and Radiotherapy, AOU Careggi, Florence. Primary tumor tissues were available in only 16 out of 53 patients. The TMB was assessed by NGS using the Oncomine Tumor mutation load assay (Thermofisher scientific) detecting and annotating low frequency somatic variants from 409 genes. DNA (10 ng) from FFPE tissues will be used for library preparation. For the sequencing reaction the Ion S5 instrument (Thermofisher scientific) was used. Data analysis was carried out using the Ion Reporter Software 5.10 analysis workflow providing the normalized Mutation Load (mutations/Mb) and annotations of low frequency cancer driver variants.

Results and Discussion (Preliminary results)

Unfortunately, 8 of the 16 FFPE samples collected showed no neoplastic cells after the diagnostic workflow. In the remaining cases, DNA and RNA concentrations and/or quality were low, making TMB analysis impossible.

Conclusions

Due to the low amounts of FFPE neoplastic material and the preanalytical criticisms of nucleic acid preservation, our preliminary results suggest that diagnostic bioptic tissues are extremely critical for obtaining additional information, besides diagnosis and “classical” biomarkers, such as TMB.

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P4 - Optimization of a protocol for the analysis of miR contained in EV obtained from low-volume biological fluids

REYRE M.[1], PÉCHOUX C[2], MADRY H[2], KRUPOVA K[1], DEFRENAIX P[1], TIXIER-BOICHARD M[2], BEVILACQUA C[2]

Introduction

To be able to use microRNA (miR) contained in exosomes and extracellular vesicles (EV-miR) in the diagnosis or monitoring of pathologies in animals or humans, it is necessary to consolidate the technique that allows obtaining an EV-miR profile from a small volume of starting body fluids (less than 1 mL) which, to date, remains a major technical challenge.

For this purpose, two French laboratories, a public INRAE unit (GABI including two core facilities: @BRIDGe and MIMA2) and a private company (Excilone), working in an Associated Partnership Laboratory, combined their complementary skills to propose miR signature characterizing biofluids in different species starting from low volumes of samples.

Material and Methods

Extracelluler vesicles (EV) were isolated using different methods such as sucrose gradient ultracentrifugation and size exclusion chromatography, from human and pig plasma and goat and cow milk (n=5 per group). EV-miR expression of selected miR was analyzed by qPCR (12K QS Flex, ThermoFisher Scientifics) and ddPCR (ddPCR QX200, Bio-Rad) to establish the limit of detection and the limit of quantification of each method in order to select the most appropriate one.

First, we compared qPCR and ddPCR sensitivity through the expression analysis for three miR of different levels of expression in a reference sample of bovine milk-derived EV: hsa-miR-148a (miR 1), hsa-miR-30a-5p (miR 5) and hsa-miR-103 (miR 2) considered as highly, mildly and lowly expressed, respectively.

Second, we applied the best procedure on other species samples (goat, pig, human) measuring three miR of different levels of expression specific for each species.

Conclusions

The first results show that ddPCR was the most robust and sensitive technique starting at 10 pg of material. With Ct ranging from ~29 to 35 for highly and lowly expressed miR, respectively, qPCR was not suitable for a precise and reproducible quantification of EV-miR expression, starting at low quantities of RNA.

The same protocol was then successfully applied to normalized volume (100 µl) of human and pig plasma and goat milk, thus validating ddPCR approach to profile EV-miR expression in small samples of material.

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P5 - Angiogenic profile in patients with NSCLC

DEL GAUDIO C, SIMONIS V, FILIPPELLI A, CICCONE V, BENGALA C, SCOTTI V, DONNINI S[1]

Background

Non-Small Cell Lung Cancer (NSCLC) is the most common cause of cancer death worldwide, with an estimate of 1.6 million deaths each year (WHO). Among the therapies used against NSCLC progression immune checkpoint inhibitors (anti-PD1, ICIs) have proven to positively modulate overall survival, progression free survival and objective response rate in cancer patients (Tian W. et al., 2020). However, the diagnostic use of PD-L1 expression for the treatment of NSCLC patients with ICI has not been found to be a predictive biomarker of effective response. Tumor angiogenesis with irregular and disorganized tumor vessels impairs the distribution of drugs, including ICI, and their efficacy (Filippelli A. et al., 2020). The aim of this project has been to study the expression profiles of molecular determinants of angiogenesis as potential new biomarkers of the efficacy of ICI. The study has been done in plasma (in platelets) of NSCLC patients before and during ICI treatment and at disease progression. The final aim will be to correlate these data with clinical data of treatment response.

Materials and methods

To date, 53 patients with diagnosis of non-operable advanced stage NSCLC, without history of previous or concomitant other neoplastic disease, candidates to treatment with ICI as first or second line of treatment were accrued by UOC Oncology and Radiotherapy, AOU Careggi, Florence. Blood was collected at baseline, at 2- and 4-months during treatment, and at the time of progression. The poor plasma platelets (PPP) and platelet-rich plasma (PRP) were generated by centrifugation and collected. Multiplex ELISA assay was performed to characterize platelet lysate by the quantification of main markers involved in angiogenesis and/or vessel normalization. Particularly, the concentration of Angiopoietin 2 (Ang 2), Interleukin-8 (IL-8), Vascular Endothelium Growth Factor (VEGF), Fibroblast growth factor (FGF), and Platelets Derived Growth Factor (PDGF) were assessed.

Results and Discussion

Now, a cohort of women (n=22) and men (n=31) with a mean age of about 65 years was enrolled in this clinical trial. For 18 patients, only blood samples were collected at baseline (T0), while 28, 4 and 6 samples were obtained at 2 months (T2) and 4 months (T4) during treatment and at the time of progression (Tp), respectively. For the first 19 patient whose samples were available at T0 and T2, a multiplex ELISA assay was performed to assess the concentration of Ang 2, VEGF, FGF, PDGF, and IL-8 in platelet lysate. Preliminary data confirmed that VEGF is the main angiogenic marker carried by platelets. The level of PDGF seems to increase with the onset of progression. On the contrary, IL8 and Ang2 showed very low expression in platelet. The modulation of these two markers needs to be further analyzed, but so far, no uniform trend has been assessed between patients in the two periods analyzed.

Conclusion

The preliminary data will be integrated with molecular (TMB and cfDNA), histological and clinical data using the multi omics data platform to validate the role of platelets as a potential biomarker of angiogenesis and response to immunotherapy. The proposed approach would allow to identify new reliable circulating and tissue markers as predictive factors of activity and efficacy of ICI in patients with advanced NSCLC.

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P6 - In Situ Single-cell Transcriptomic Imaging in Formalin-fixed Paraffin-embedded Tissues with the MERSCOPE® Platform

HE JL, WIGGIN T, GENSHAFT AS, PETRENKO N, FERNANDEZ N, CHEN R, COLBERT L, EMANUEL G, HE J.

Abstract

Formalin-fixed paraffin-embedded (FFPE) tissues are the most widely used clinical sample types in histology and molecular diagnosis, but these samples are often challenging for single-cell transcriptomic analysis due to RNA degradation and protein crosslinking. A spatial transcriptomics technique with high detection efficiency and single molecule resolution is required in order to accurately profile the gene expression in FFPE samples in situ. Vizgen's MERSCOPE platform, built on multiplexed error robust in situ hybridization MERFISH technology, directly profiles intact tissue's transcriptome with subcellular spatial resolution. Here, we demonstrate the FFPE MERSCOPE workflow in tissues from 10 mouse and human samples, including archival clinical samples. In each sample, hundreds of thousands of cells were captured with >100 million transcript counts, generating detailed spatial transcriptomic data for the profiled genes in each sample. A comparison of FFPE and matched fresh frozen samples indicated that the FFPE workflow performs similarly in detection efficiency as compared to the fresh frozen protocol. We further demonstrated the MERSCOPE FFPE workflow is compatible with protein imaging by performing simultaneous protein-based cell boundary staining with MERFISH to accurately profile gene expression and map cell types in archival clinical human samples. Finally, we constructed a spatially resolved single cell atlas across eight major tumor types, mapped and cataloged different cell types within the tumor microenvironment and systematically characterized the gene expression among cells. This study demonstrates the potential for spatially resolved transcriptomic profiling of FFPE samples at single cell level to contribute to a wide range of biomedical research areas, including many applications to study human diseases.

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P7 - Translatome vs. Transcriptome on brain tissue to unveil memory-induced genes

HELBLING JC[1], GINIEIS R, MONTALBAN E, TRIFILIEF P, FERREIRA G, MOISAN MP

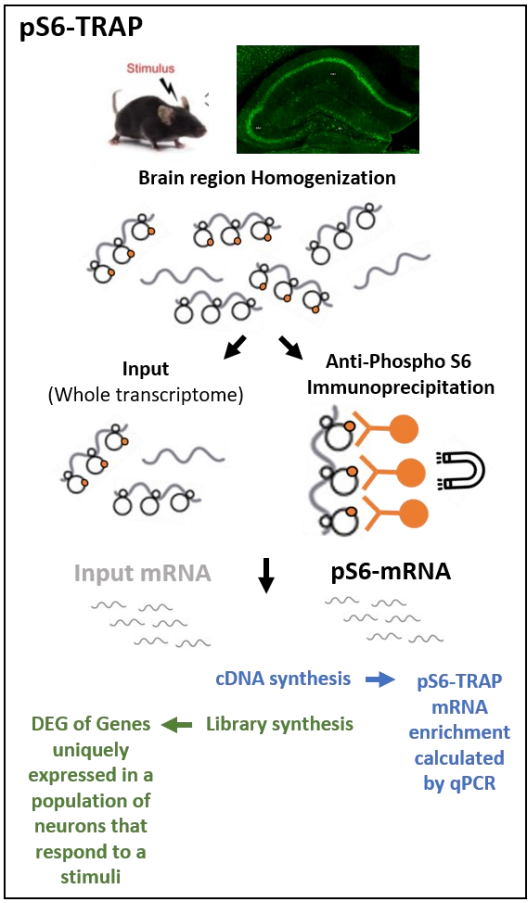
Introduction

Long-term Object Recognition Memory (ORM) depends on training-induced gene synthesis leading to protein expression in area CA1 of the hippocampus. More generally, memory formation involves mRNA for immediate early genes (IEG), transcription factors and chromatin modifiers. The purpose of this study was to identify genes modified in the hippocampus one hour after ORM training using two strategies. We first used raw RNA sequencing with Laser Capture Microdissection (LCM) to decipher gene expression specifically in CA1. In parallel, on the whole hippocampus, we used RNA sequencing on purified mRNA using phosphorylated ribosome capture, ie Translating Ribosome Affinity Purification with phosphorylated ribosomal protein S6 (pS6-TRAP), in order to enrich our preparation with hippocampal cells activated by ORM training.

Material and Methods

- Test mice were euthanized and either the whole hippocampus or CA1 region were isolated 1h after ORM training. Control mice were left in their home-cage and euthanized at the same time as test mice.
- Transcriptome: raw RNAseq on mRNA from CA1 region was isolated by LCM
- Translatome: RNAseq on pS6-TRAP mRNA was purified from total hippocampus

pS6-TRAP mRNA enrichment was estimated by qPCR.
Differentially Expressed Genes (DEG) uniquely in a population of neurons modified by the task was obtained by RNAseq and bioinformatic analysis.



Results and Discussion

Raw RNAseq allowed the identification of genes modified by ORM training by comparing hippocampal CA1 mRNA expression from ORM and home-cage groups (DEG).
RNAseq on pS6-TRAP purified hippocampal mRNA provided a stronger contrast for DEG associated to memory formation and a relevant enrichment of well-known genes induced during this memory phase (Fos, Arc). Compared to raw RNAseq, pS6-TRAP RNAseq gave us information about the cellular types involved in memory formation.

Conclusions

This study provides evidence that the pS6-TRAP strategy improves the identification of memory-associated genes and reveals specific links between molecular diversity and brain area functions.

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P8 - V-TRAP: Viral based Strategy to Isolate Cell Type Population Specific mRNA expression

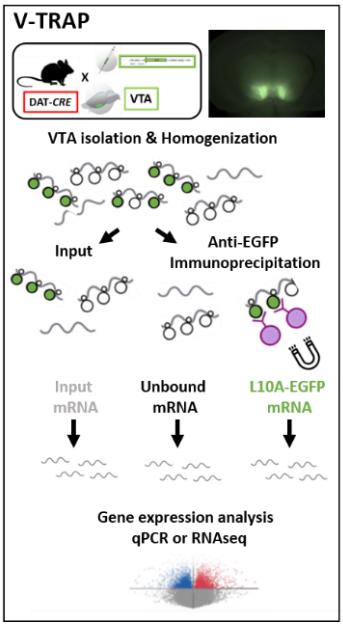
HELBLING JC[1], MONTALBAN E, HARDT L, VALJENT E, TRIFILIEFF P

Introduction

The central nervous system is characterized by large cell heterogeneity as well as remarkable flexibility in gene expression that modulates the correct action in response to a wide variety of environmental stimuli. Multicellularity represents a serious limit to study the alterations in gene regulation within individual cell types. The recent development of BAC-TRAP technology helped to overcome this problem. The BAC-TRAP (translating ribosome affinity purification) technology consists in a rapid affinity purification strategy for the isolation of translated mRNA from genetically targeted cell types. In BAC transgenic mice, a fluorescent EGFP is fused to the N-terminus of the large subunit ribosomal protein L10a and inserted under the control of the promoter of a gene of interest. The major advantage of the BAC-TRAP is that it allows studying the changes within an identified cell population in response to a challenge. Here, to avoid possible issues linked to the strength of transgenic promoter and in order to isolate specific subregions of interest, we optimized a viral strategy allowing the expression of the L10a-EGF construct under the control of a specific Cre driver mouse line.

Material and Methods

- pAAV-FLEX-EGFPL10a vectors were locally injected in areas of interest, followed by EGFP immunoprecipitation V-TRAP mRNA purification (1) from D2 receptor-expressing dopaminoceptive neurons in the dorsal striatum (DS) using DRD2-Cre transgenic mice and (2) from dopaminergic neurons in the ventral tegmental area (VTA), using DAT-Cre transgenic mice.
- Fold expression relative to Input or unbound fractions were analyzed by quantitative real-time PCR (qPCR) to validate Cell type mRNA enrichment and depletion.



Results and Discussion

V-TRAP in the DS allowed a relevant enrichment for several specific genes expressed in targeted cells. qRT-PCR analysis revealed a strong *Drd2* mRNA enrichment for dopaminoceptive neurons and depletion of *Drd1* mRNA which represents 50% of dorsal striatum neurons and depletion for other cell type markers (*Gfap* for astrocyte cells).
V-TRAP mRNA purification from dopaminergic neurons in the VTA was more challenging due to the depth and small size of the brain area. V-TRAP in VTA involved an optimization and comparison protocol in order to use only one mouse and avoid mice pooling in the aim of obtaining enough mRNA materials for further analysis ie. qPCR or RNAseq analysis.

Conclusions

With V-TRAP we can now profile the entire translatome of subpopulations of interest from small brain areas using only one animal. Purified mRNA material will be further used for RNAseq analysis to characterize the translatome landscape of dopaminergic neurons of the VTA.

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P9 - Extracellular vesicles within the living tumor microenvironment provide a new class of functional communication information: Implications and Applications for therapeutic monitoring.

HOWARD M, GADE P, LUCHINI A, KASHANHI F, LIOTTA L[1]

Abstract

Tumor interstitial fluid (IF) extracellular vesicles (EVs) are a new category of real-time biomarkers reflecting the ongoing cell-cell communication within the tumor microenvironment. We have developed a highly reproducible methodology for harvesting and characterizing resident EVs within the living tumor IF (Howard et al, 2022). In this study, we explored the cell type of EV origin within tumor microenvironment, following immune perturbation of the tumor microenvironment. Tumor IF contains an abundance of the full EV molecular repertoire produced by the various cell types within the tumor microenvironment. IF EVs contain information not present within the cultured cells. Molecular analysis of the IF EVs by proteomics can distinguish the cell source of the EV. A new class of mitophagy EVs were discovered and characterized by the presence of PINK1, the key mitophagy initiating signal protein. Secretory mitophagy is a new survival mechanism that tumor cells can utilize to survive in the face of oxidative stress or high metabolic demand that is confirmed to occur in vivo and in vitro. Tumor and immune suppressors, p53 and PD-L1, bind directly to PINK1 and are exported within the secretory mitophagy EVs in proportion to the cellular level mitophagic stress. The IF EVs of Tumors treated with immunotherapy (anti-PDL1) exhibit enhanced abundance of EVs derived from immune cells and stromal cells (FDR<0.0001). Cell surface markers from different cell types were correlated to the EV marker presence within the IF. Therefore, IF EVs constitute a new way to probe the in vivo state of tumor immunotherapy. Tumor mitochondrial stress can be monitored in vivo by characterizing the PINK1+ EVs within the tumor IF. This constitutes a new approach for longitudinal monitoring of cancer therapeutic efficacy without full surgical tumor biopsy or fine needle aspirate. Secretory mitophagy constitutes a new cancer therapeutic target.

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P10 - CTC deep profiling using a label-free enrichment and isolation workflow followed by single cell 3´RNA sequencing

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Abstract

Circulating tumor cells (CTC) can be used for detection, prognosis, and monitoring of solid cancers. However, isolation and characterization of CTC remains a challenge due to several limiting factors: (1) Rarity - there are few CTC per milliliters of blood; (2) representativity - CTC are about one in a billion peripheral blood cells; (3) heterogeneity - CTC display high variability within and between different types of cancers and differentially express specific biomarkers; and (4) robustness - CTC are highly resistant cells and regular molecular biology protocols may fail. Cellenion, in partnership with Vortex Biosciences and the Centre Léon Bérard, has developed a label-free protocol to isolate and analyze CTC at single transcriptomic level. The workflow combines (1) the VTX-1® Liquid Biopsy System, a microfluidic label-free platform enabling enrichment of CTC based on their physical properties; (2) The cellenONE®, a highly accurate image-based single cell printing and liquid handling platform; (3) a modified cellenCHIP 384 3'RNAseq kit, a miniaturized plate-based gene expression kit allowing nanoliters reactions and (4) the cellenPIPE, a dedicated cloud-based analysis solution. The procedure has been thoroughly optimized to maximize recovery and purity rates on four cancer cell lines from different cancers spiked in blood from healthy donors. The protocol has then been validated for transcriptomics. The workflow has finally been applied to analyze several patient samples suffering from non-small cell lung cancer (NSCLC) stage III & IV, allowing the establishment of CTC expression profile, and a correlation with cell pictures and morphologies acquired by the cellenONE.

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P11- A first insight into the miRNA role as messengers between calf and cow in the Piedmontese cattle breed

CHESSA S, MORETTI R[1], SARTORE S, CUCCATO M, CANNIZZO FT, SACCHI P

Introduction

Colostrum has both a nutritional and protective role, thanks to its immunoglobulins, minerals, vitamins, growth factors, and immune cell content. It is also rich in microRNA (miRNA) (Van Hese et al., 2020), mainly acting on genes involved in the regulation and development of the immune and intestinal system of the calf, thus suggesting their role as messengers. This paper shows the first results of a project aiming to analyse miRNA expression in colostrum of the Piedmontese cows and in blood of their new-borns in the first days after calving.

Material and Methods

The pluriparous Piedmontese cows of the teaching farm of the Department were involved in the project. Both colostrum and blood were collected at different time points. MiRNA from the first three cows and calves were extracted from colostrum and blood using the Maxwell® RSC system. A library was prepared using the TruSeq® Small RNA Library Prep Kit and was run on the MiSeq instrument (Illumina). Sequencing results were analysed using the MiRDeep2 of the Galaxy web platform.

Results and Discussion

MiRNA extractions and the related library gave good quality products as shown by the Small RNA kit and DNA High sensitivity kit of the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), respectively.

Conclusions

A deeper knowledge of miRNA expression patterns in colostrum and blood of the new-borns will allow for a better understanding of the origin, regulation and function of miRNA in mother-calf communication and to verify whether it is possible to promote the health of calves and improve their neonatal development by optimizing the uptake of miRNA.

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P12 - Characterizing Tumor Heterogeneity and Cell Diversity Across Multiple Cancer Types

PATTERSON B, CHEN CY, FERNANDEZ N, KAUSHAL S, HE J, COLBERT L.

Abstract

For oncologists, the spatial information embedded in the heterogeneous tumor microenvironment plays a critical role in assessing patient prognosis. Vizgen's MERSCOPE® Platform is built on multiplexed error-robust fluorescence in situ hybridization (MERFISH) technology and enables the direct profiling of the spatial organization of patient tumors with sub-cellular resolution, filling an important gap in previous iterations of transcriptomic evaluation. Here, we present a 500-gene pre-designed PanCancer Pathways Panel to comprehensively assess the genomic and cellular alterations found across diverse tumor types in human clinical samples using the MERSCOPE® Platform. We demonstrate the ability of the PanCancer Pathways Panel to spatially profile gene expression across multiple tumor types, including breast, colon, prostate, ovarian, lung, and liver cancer. Comparisons between experiments across multiple cancer types in fresh-frozen (FF) and formalin-fixed, paraffin embedded (FFPE) preservation formats illustrate the robustness and reproducibility of this panel. To investigate how individual cell types in each tumor are dysregulated by cancer, we compared differentially expressed genes as detected by the MERFISH panel from normal and cancerous liver samples. We identified dysregulation in the WNT, NOTCH, and TGFB signaling pathways in the hepatocellular carcinoma samples, which are pathways known to play central roles in the development and progression of hepatocellular carcinoma. These results demonstrate the power of the MERSCOPE® Platform and the PanCancer Pathways Panel to generate individualized, accurate cell atlases from patient-derived tumors and to enable further insights into the relationship between genomic profiles, dysregulated pathways, and disease phenotypes. Such network-centric approaches are critical for assisting with identifying genotypic causes of diseases, classifying disease subtypes, and identifying drug targets.

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