Single Cell Europe Conference

September 19 – 21, 2018

BIOCEV, Vestec by Prague, Czech Republic

BOOK OF ABSTRACTS

www.singlecell2018.eu

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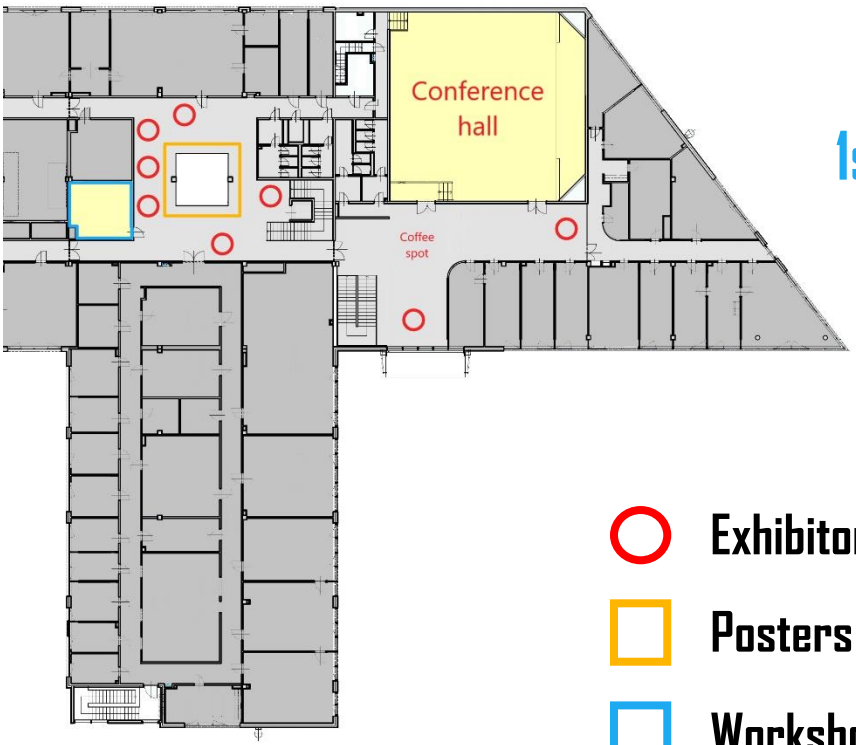
BIOCEV

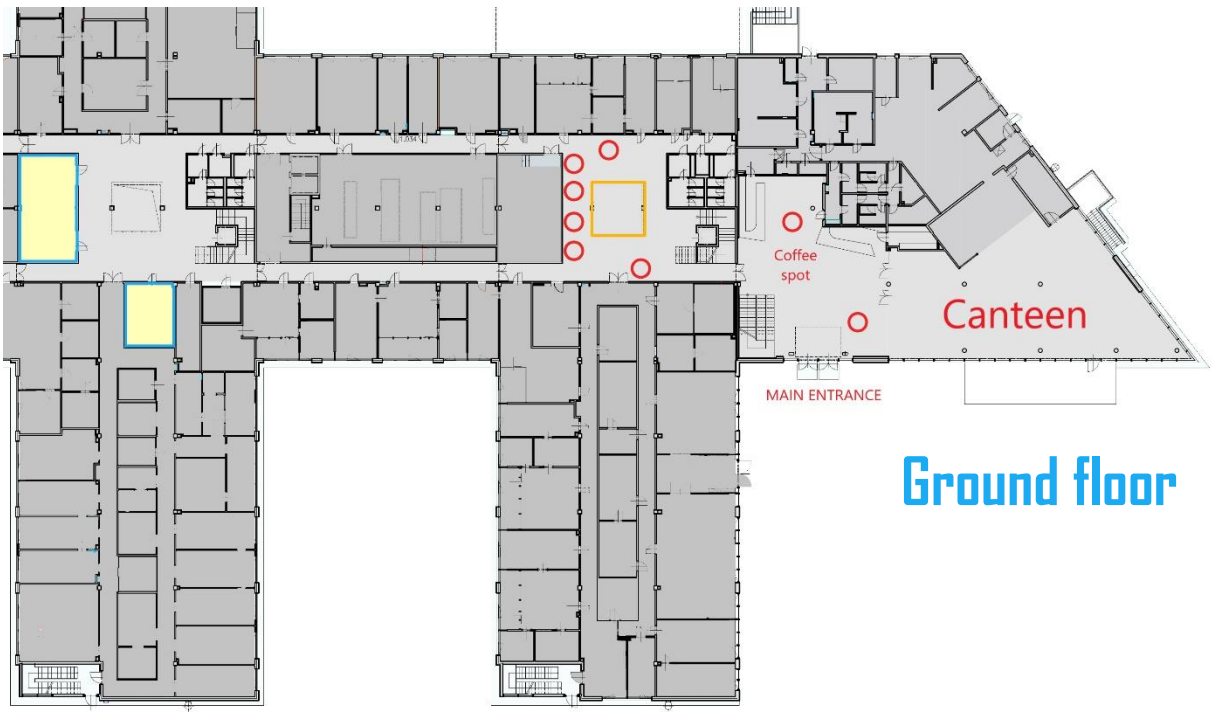


1st floor

Exhibitors

Posters

Workshops



Ground floor

Book of Abstracts

Single Cell Europe 2018

September 19 – 21, 2018

BIOCEV

Vestec, Czech Republic

The local organising committee:
Alice Mášová
Veronika Kašparová
Eva Rohlová
Lucie Langerová
Filip Franko
Radek Šindelka
Lukáš Valihrach
Pavel Abaffy
Ondřej Smolík
David Švec
Peter Androvič
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Ravindra Naraine
Šárka Benešová
Daniel Kraus

Acknowledgement

We would like to offer our heartfelt appreciation for all the hard work contributed by the many individuals and organizations that helped to make this workshop and conference a success. The organizers would like to especially express an acknowledgement to Prof. Pavel Martásek, M.D., D.Sc., the BIOCEV director, and his team at BIOCEV for their support. We would also like to thank the Institute of Biotechnology CAS, v. v. i. (especially doc. RNDr. Jana Pěknicová, CSc.) for their continued support. We are also grateful for the tireless work that the members of the IBT's Gene Expression group have contributed to ensuring the success of the conference. Thank you to our invited speakers, sponsors and all the attendees who all contributed to making this conference an enjoyable and scholastic achievement.

BIOCEV

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Contents

Welcome to Single Cell Europe 2018

Dear colleagues,
dear researchers,
dear company representatives,

It is with great pleasure I welcome you to the first Single Cell symposium in central Europe organized at the beautiful campus BIOCEV of the Czech Academy of Sciences and Charles University. The interest for the meeting has been great and I am very happy to welcome many prominent speakers, innovators and industry leaders as well as forthcoming young researchers and students. I am confident we will all enjoy most exciting scientific talks, while we also will have great opportunities to socialize and meet old and make new friends, catalyzed by the world's best beer!
Wishing you all a most fruitful meeting!

Mikael Kubista
Chairman of the Single Cell Europe Conference

Practical information

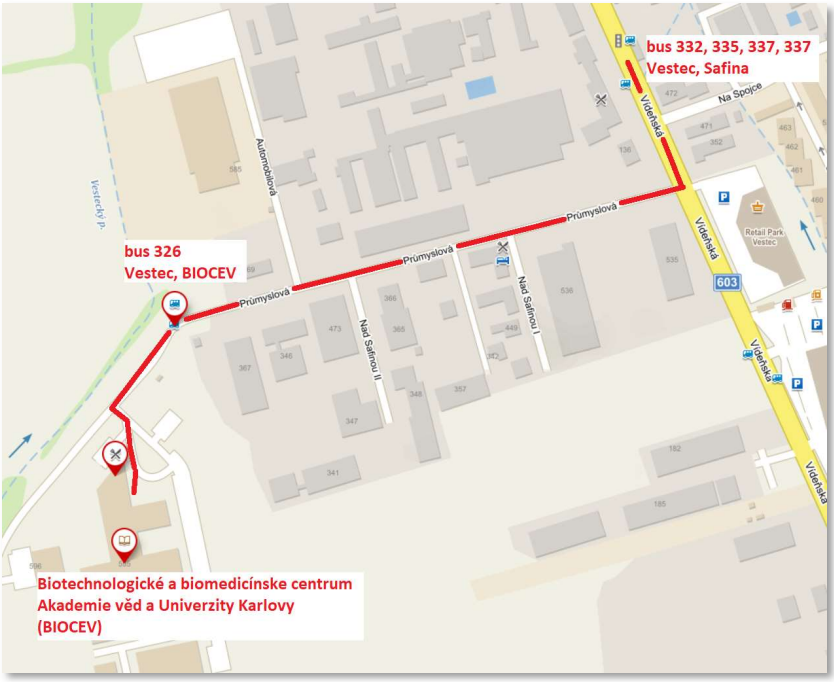
How to get to the conference location

BIOCEV
Průmyslová 595
252 50, Vestec
Czech Republic

GPS coordinates:
49.9813803N
14.4874594E

Option 1
1. Metro C - Opatov station
2. Bus No. 326 to the stop Vestec, BIOCEV
3. From the bus stop, walk to the BIOCEV building (170 m).

Option 2
1. Metro C - Budějovická station
2. Bus No. 332, 335, 337, 339 to the stop Vestec, Safina
3. 100 m forward, turn right to Průmyslová street
4. Enter the area of BIOCEV center at the end of the street



Public transport

Just outside airport you find bus stop "Letiště Václava Havla" (Václav Havel airport). Take bus No. 119 to the metro station "Nadraží Veleslavín" (approx 10 min.). "Nadraží Veleslavín" is on the metro line A- green. From "Nádraží Veleslavín" take the metro to the "Muzeum" station. At "Muzeum" change to metro line C (red) – direction "Háje" and exit at station "Opatov". From "Opatov" take bus No. 326 in the direction Jesenice to the stop „Vestec, BIOCEV" (17 min.).

If you want to go from the airport to a hotel downtown start at the station "Letiště Václava Havla" (Václav Havel airport). Take the bus No. 119 to the metro station "Nádraží Veleslavín" and from there continue with metro line A (green) downtown.

Vestec is in the periphery area of Prague. If you travel from the city Center or the airport to Vestec, buy a 40 CZK ticket. This ticket is valid for 120 minutes after checking in. Stamp it in the ticket machine first time you enter metro or bus.

We recommend the mobile application "PID info", for the convenient navigation of the public transportation routes within the Prague City. Just enter your destination station and the app will indicate all possible public transportation options including connections. It also calculates the expected cost of the journey and provides the possibility to purchase an SMS public transport ticket. Additionally, it shows you on the map all locations of ticket vending machines.

Mobile application: https://pid.cz/en/mobile-app/

Venue

BIOCEV is a research center in the heart of Europe. The two most important institutions in Czech Republic, the Czech Academy of Sciences and Charles University, have teamed up to create a scientific centre of excellence with more than 25,000 square metres of new laboratories, equipped with state-of-the-art technologies. The institute is focused on education, R&D and technology transfer in the fields of Molecular Biology, Genetics, Virology, Medical Chemistry and Structural Biology.

Catering

The BIOCEV canteen is operated by the well-known gastronomic and catering company Perfect Canteen. Lunches will be served in the BIOCEV canteen on the ground floor of the BIOCEV. The coffee breaks will be served in the BIOCEV's foyer on the ground floor and in the front of the Conference hall on the first floor.

Badges

All registered participants will receive a name badge upon registration. Delegates are kindly requested to wear their name badge when attending the conference as this will allow you entry. Only participants with name badges will be admitted to the conference hall and lecture rooms.

Name badges have been colour-coded as follows:

Speakers - Red

Sponsors - Black

Participants - Blue

Organisers – Green

Social events

Beer event

- Wednesday, September 19

- Starts at 18:00 at BIOCEV canteen

The Beer event reception will include some light refreshments. The event is informal and offers a great opportunity to meet colleagues in a relaxed setting.
The Muflon light lager 11° beer, winner of the prestigious international GOLDEN BEER PEAK 2017 competition, will be served.

Boat trip in Prague City Center with dinner

- Thursday, September 20

- Starts at 19:00 at the Dvořák's waterfront – at The Francis, Prague

If you are registered for the cruise on Thursday, a bus leaves from the BIOCEV venue at 18:00 to take you to the boat. If you go directly to the boat, you will find the boat at the Dvořák's waterfront – at The Francis ("Na Františku"). Please be there on time and bring your boat trip voucher you received at the registration!

GPS coordinates:
50.0934119N
14.4257700E

Oral presentations

The talks are at the Conference hall on the first floor of BIOCEV. The allocated time for a talk with discussion is 20 minutes. Invited talks are for 40 minutes incl. discussion. The equipment for the presentation (PC, microphones and a laser pointer) is provided. Speakers shall bring their presentations on a USB to technical assistant in the conference hall ahead of the session when they present.

Poster session

The poster sessions take place on Thursday and Friday, 20 and 21 September, during Coffee and Lunch breaks. The participants are encouraged to set up their posters early in the morning (just after the registration). The recommended poster format is A0, although smaller posters can also be mounted. Thumb tacks will be provided to hang the posters.
All poster presenters are enrolled in the Best Poster Award, sponsored by the International Journal of Molecular Sciences. Conference participants and invited speakers vote for the best poster. Every participant receives one poster vote, which can be used to support his/her favourite poster. By the end of the poster session, the tickets shall be deposited in a ballot box placed at the door to the conference hall. The results of the poster voting will be announced before the closing of the conference. In case of a draw, the winning poster will be selected by the speakers.

Internet connection

Internet will be provided at the BIOCEV venue for the duration of the conference. Detailed instructions on how to connect to the internet are provided in your conference bag.

Program changes

The organizers do not assume liability for any changes in the programme due to external or unforeseen circumstances.

Taxi

Distance from Václav Havel airport to the Prague City Centre is approximately 20 km and to the Single Cell Europe Conference 2018/BIOCEV distance from the airport is approximately 30 km.

Some Prague taxi services:

TICK TACK taxi	+420 721 300 300	objednavka@ticktack.cz
AAA Radio taxi	+420 222 333 222	hotline@aaataxi.cz
Citytaxi	+420 257 257 257	operator@citytaxi.cz
Modrý Anděl	+420 737 222 333	online@modryandel.cz

Local contact personnel

Alice Mášová	alice.masova@tataa.com	+420 603 742 991
Eva Rohlová	eva.rohlova@tataa.com	+420 734 224 605

Program

Day 1, Wednesday 19th of September 2018

08:30	09:00	**WORKSHOP REGISTRATION**
		WORKSHOPS
09:00	16:30	• microRNA analysis • RT-qPCR Single cell expression profiling • Single cell isolation
15:45	16:40	CONFERENCE REGISTRATION and Poster setup
16:40	16:50	CHAIRMAN TALK – Mikael Kubista
16:50	17:30	**Anders Ståhlberg -**Tumor heterogeneity and liquid biopsies: The use of single-cell and single-molecule analyses in breast cancer and liposarcoma
17:30	18:00	**Siegfried Hauch -** An easy method for CTC single cell isolation and subsequent molecular profiling
18:00		**BEER EVENT** with music

Day 2, Thursday 20th of September 2018

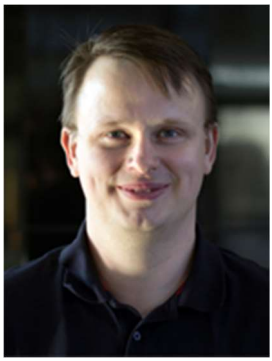
08:00	09:00	**REGISTRATION and Poster setup**
09:00	09:10	**WELCOME – Pavel Martásek,** BIOCEV director
		Session 1
09:10	09:50	**Marc Wadsworth II -** Approaching the Immune System as an Interacting Ensemble of Cells
09:50	10:30	**Martin Hemberg -** Computational tools for analyzing large collections of scRNA-seq datasets
10:30	10:45	**10x GENOMICS presentation**
10:45	11:20	COFFEE BREAK
		Session 2
11:20	11:40	**Catherine Alix-Panabières** - Molecular and functional characterization of circulating tumor cells in carcinoma patients
11:40	12:00	**Lukáš Valihrach** -Single cell analysis of oligodendroglial lineage cells during post-ischemic regeneration
12:00	12:20	**Evi Lianidou -** Liquid Biopsy: The clinical potential of single Circulating Tumor Cell analysis
12:20	12:40	**Leon Terstappen** - Tools to identify and characterize single Circulating Tumor Cells
12:40	13:00	**Nikolas Stoecklein** - Molecular Characterization of CTCs detected with the CellSearch System
13:00	13:20	**Katarína Kološtová** – Character of circulating tumor cells as a part of liquid dynamic medicine in oncology
13:20	15:20	LUNCH / **POSTER SESSION**
		Session 3
15:20	16:00	**Fuchou Tang** - Deciphering the gene regulation networks in human early embryos
16:00	16:40	**Norman Dovichi -** Proteomics of early development: the assembly of the vertebrate brain
16:40	17:00	**Radek Šindelka** - Subcellular transcriptome and proteome analyses to reveal body plan determinants of early development
17:00	17:10	**Samuel Bouvet -** SMARTer Solutions for Single-Cell Transcriptomics and Genomics
17:10	18:00	REFRESHMENT BREAK
18:00		BUS FROM BIOCEV TO THE CITY CENTER**, BOAT TRIP with dinner**

Day 3, Friday — 21st of September 2018

		Session 4
09:00	09:40	**Vessela N. Kristensen -** Tracing the origin of disseminated tumor cells in breast cancer using single-cell sequencing
09:40	10:00	**Pamela Pinzani -** Molecular analysis of single circulating tumor cells from cancer patients
10:00	10:20	**Mikael Kubista -** Two Tailed-PCR for precision diagnostics
10:20	10:40	**Christer Ericsson -** Screening circulating tumor cells as a non-invasive cancer test in 3,388 individuals from high-risk groups (ICELLATE2)
10:40	11:00	**Guilhem Tourniaire -** cellenONE X1 : A versatile platform for single cell isolation
11:00	11:15	**DOLOMITE presentation**
11:15	11:45	COFFEE BREAK
		Session 5
11:45	12:25	**Pascal Barbry** - A new functional genomics approach to understand the human airways at a single cell level
12:25	12:45	**Ilaria Santeramo -** Single cell Viral Copy Number analysis using ddPCR
12:45	13:05	**Peter Karlberg** - Celsee Genesis: Comprehensive Single Cell Preparation
13:05	13:35	**Jennifer Chew** – SureCell ATAC-Seq: A new platform for Single Cell ATAC-seq using Bio-Rad's droplet digital technology
13:35	15:20	LUNCH / **POSTER SESSION**
		Session 6
15:20	16:00	**Michael W. Pfaffl** - Exosomal microRNA Biomarkers Signatures in Clinical Diagnostics
16:00	16:20	**Pavel Vodička -** Search for prognostic and predictive markers in sporadic CRC
16:20	17:00	**CLOSING SUMMARY / BEST POSTER AWARD CEREMONY by Mikael Kubista**

Talks

Anders Ståhlberg



Gothenburg University, Sweden
http://cancercenter.gu.se/

Anders Ståhlberg, Associate Professor, is working as principal investigator at the Cancer Center, Sahlgrenska Academy, University of Gothenburg in Sweden. He has a PhD in molecular biotechnology and has two post-doc periods working with human embryonic stem cells and tumor biology. Anders primary research interest is to understand molecular mechanisms in tumor initiation, tumor development and stem cell differentiation. The research is focused on breast cancer and sarcomas. He has developed several strategies for gene expression profiling and rare molecule analysis, especially at the single-cell level.

T1: Tumor heterogeneity and liquid biopsies: The use of single-cell and single-molecule analyses in breast cancer and liposarcoma

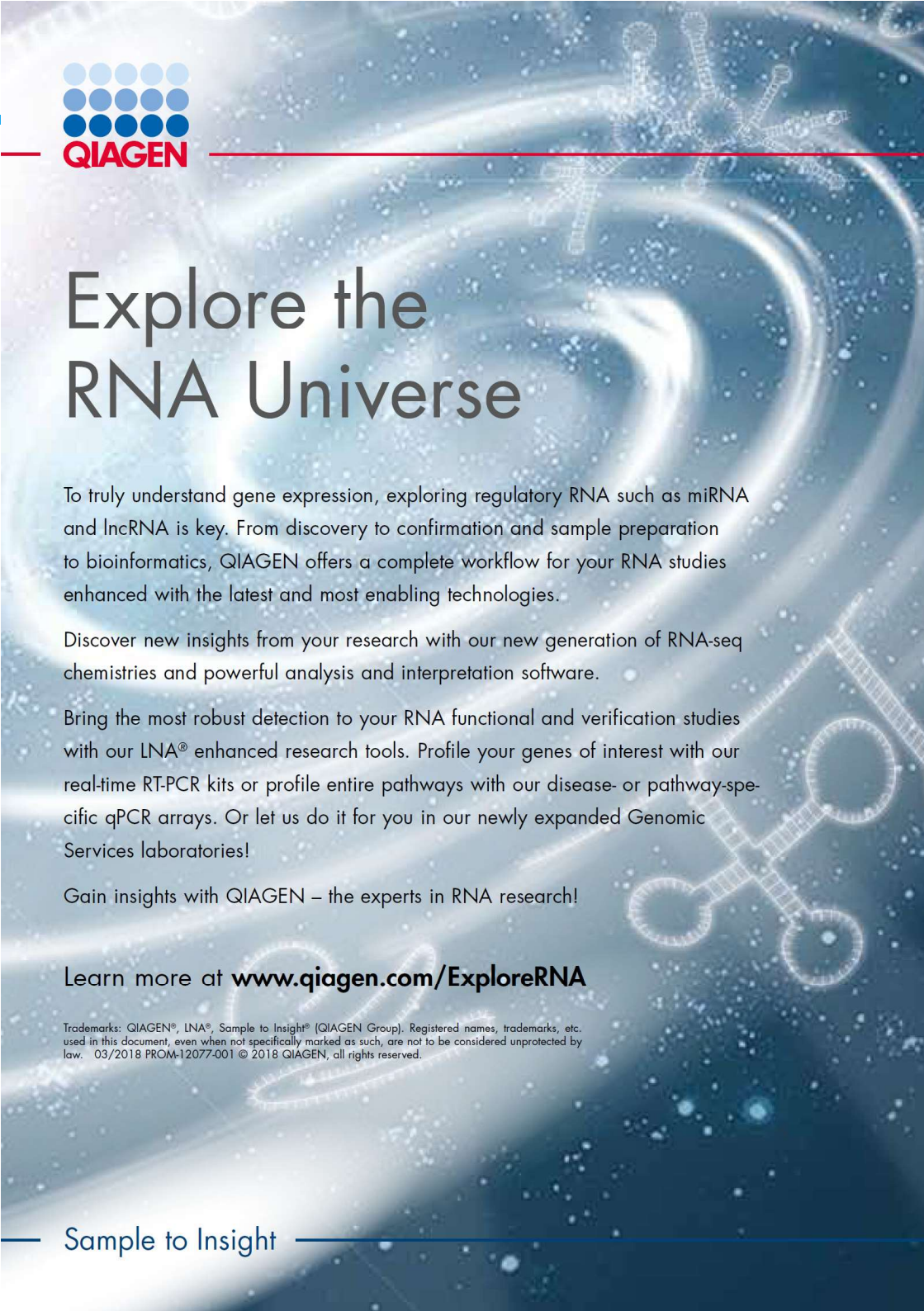
Anders Ståhlberg

Introduction: Tumors are heterogeneous and contain multiple cell types with different phenotypes. Characterization of individual tumor cells enables improved understanding of molecular mechanisms in tumor development. Detection of single tumor DNA molecules also allows early detection of cancer.
Aims: (i) To define tumor subpopulations in breast cancer and liposarcoma at molecular level. (ii) To develop and apply diagnostic tools to detect and monitor cell-free tumor DNA at allele frequencies < 0.1%.
Materials and Methods: We have used functional cell assays combined with single-cell gene expression profiling to define tumor subpopulations in breast cancer and liposarcoma. We have also developed, SimSen-Seq, an ultrasensitive sequencing approach that is suitable for cell-free tumor DNA analysis in liquid biopsies.
Results: Here, we show how cell proliferation, cancer stem cell properties and microenvironment contribute to intratumor heterogeneity in breast cancer and liposarcoma. We also demonstrate the use of SimSen-Seq in several cancer applications.
Conclusion: Single-cell and single-molecule analyses provide new means to decipher tumor heterogeneity and improve cancer diagnostics.

T2: An easy method for CTC single cell isolation and subsequent molecular profiling (QIAGEN)

Siegfried Hauch

Counting of circulating tumor cells in the blood of cancer patients is meanwhile widely accepted to provide important prognostic information but also their molecular characterization is realized as an important need to better understand tumor biology, resistance mechanisms and metastasis formation. Especially in this context important CTC phenotypes ranging from cells that undergo different states of epithelial to mesenchymal transition (EMT) to tumor stem cells can be identified. Consequently, single cell CTC research is now getting more and more important taking into consideration that many different CTC phenotypes with different clinical meanings may be found in the circulation in parallel and need to be analyzed separately to get a full picture of the cancer progress. However, single cell CTC research so far appears challenging and usually requires sophisticated instrumentation. Aiming to provide a simple workflow with low complexity in that context we successfully combined QIAGENs QIAscout single cell isolation platform with the AdnaTest "combination of combinations principle (COCP)" CTC enrichment technology to purify single CTCs from blood samples for subsequent transcriptional profiling and genomic research.


QIAGEN
Explore the
RNA Universe
To truly understand gene expression, exploring regulatory RNA such as miRNA and lncRNA is key. From discovery to confirmation and sample preparation to bioinformatics, QIAGEN offers a complete workflow for your RNA studies enhanced with the latest and most enabling technologies.
Discover new insights from your research with our new generation of RNA-seq chemistries and powerful analysis and interpretation software.
Bring the most robust detection to your RNA functional and verification studies with our LNA® enhanced research tools. Profile your genes of interest with our real-time RT-PCR kits or profile entire pathways with our disease- or pathway-specific qPCR arrays. Or let us do it for you in our newly expanded Genomic Services laboratories!
Gain insights with QIAGEN – the experts in RNA research!
Learn more at www.qiagen.com/ExploreRNA
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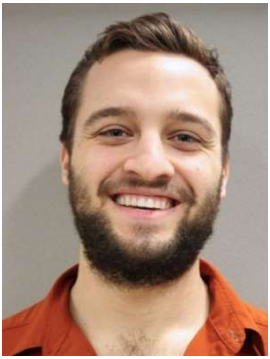
Sample to Insight

Marc H. Wadsworth II



MIT - IMES - Shalek Lab, Cambridge, USA
http://imes.mit.edu/

Marc H. Wadsworth II received his B.S in Chemistry Cum Laude from Miami University of Ohio in 2014 and is currently pursuing a PhD in Chemistry at MIT with Prof. Alex K. Shalek's lab. His research interests center on the development and application of new technologies to elucidate how cells and their interactions lead to system-level functions in health and disease. Illustratively, Marc was a co-developer of Seq-Well, a massively-parallel single-cell RNA-Seq platform for low-input clinical samples. He has since applied Seq-Well and related methods to examine, at unprecedented resolution, the ecosystems of M. Tuberculosis and Malaria infections.

T3: From the Bottom Up: Studying Immune Tissues and Their Responses One Cell at a Time

Marc H. Wadsworth II

The heterogeneity in the immune system is critical for protecting the host against a broad range of threats, and can most clearly be seen during dynamic processes such as antigenic response. In recent years, we have witnessed transformative and intersecting advances in nanofabrication and molecular biology that enable deep profiling of low-input samples. These afford new and exciting opportunities to study heterogeneity in immune responses, starting from the level of the single cell, with the potential to fundamentally advance our understanding of systems-level immune regulation in health and disease. Illustratively, I will discuss how we can leverage single-cell genomic approaches – and, in particular, single-cell RNA-Seq – to explore the extensive functional diversity between immune cells, and uncover, from the bottom-up, distinct cell states and their molecular drivers. Moreover, I will present new experimental strategies for translating these powerful techniques to the clinic to identify therapeutically and prophylactically relevant intra- and inter-molecular circuits in the context of M. Tuberculosis and more.

Martin Hemberg

Sanger Institute, Cambridge, England
https://www.sanger.ac.uk

Martin Hemberg is a group leader at the Wellcome Trust Sanger Institute where his primary research interests are in quantitative models of gene regulation and gene expression. Currently, the main focus of the group is to develop methods and models for computational analysis of high throughput sequencing data obtained from single-cell experiments that will allow researchers to address novel biological questions.

T4: Computational analyses of large collections of scRNA-seq datasets

Martin Hemberg

As sequencing costs continue to drop and experimental platforms for carrying out single-cell experiments improve, the number of single-cell RNA-seq (scRNA-seq) datasets available is increasing rapidly. A large international consortium is currently working towards a Human Cell Atlas and it will contain multiple scRNA-seq datasets, with the total number of cells most likely >100 million. For this resource to be useful, it is important that researchers are able to carry out fast and efficient queries. Here, I will present two novel computational methods, scmap and scfind, that make it possible to quickly search large collections of scRNA-seq datasets. scmap is the single-cell equivalent of the popular BLAST algorithm for searching nucleotide or amino acid databases. As such, it allows the user to compare cells from a query sample to an annotated reference database to identify the closest matching cell and cell-type. In addition to searching by cells, an important way of accessing scRNA-seq data is to search based on a list of genes (e.g. from a GWAS study or binding targets identified from a ChIP-seq experiment). scfind makes it possible to quickly retrieve the set of cells that express a given gene. As an example application, it is demonstrated that scfind can define marker genes against a large background of other cells. Furthermore, we demonstrate how a quantization approach can be used to store approximate expression values with very small overhead. The approximate expression values can be used for a search function that allows the user to identify the cells that provide the 'best' match to an arbitrary list of genes.

Catherine Alix-Panabières

Centre Hospitalier Universitaire de Montpellier, France
https://www.cancer-id.eu/

Dr Catherine Alix-Panabières received her PhD degree in 1998 at the Institute of Virology, University Louis Pasteur, in Strasbourg in France. In 1999, she moved to Montpellier where she did a postdoctoral research in the Department of Immuno-Virology of the University Medical Centre of Montpellier, France. During this last decade, Dr Alix-Panabières has focused on optimizing new techniques of enrichment and detection of viable disseminating tumor cells in patients with solid tumors. She is the expert for the EPISPOT technology that is used to detect viable tumor cells in the peripheral blood and the bone marrow of patients with breast, prostate, colon, head & neck cancer and melanoma. This technology has been recently improved to detect functional CTCs at the single cell level and is called EPIDROP. In 2010, she achieved getting a permanent position at the Hospital and at the Faculty of Medicine of Montpellier (MCU-PH), a wonderful mixture of giving teaching lessons to medical students on Cancer Biology in combination of developing this field of tumor cell dissemination at the hospital for the cancer patients, leading strongly translational clinical research. As an associate professor, she recently became the new director of the Laboratory of Rare Human Circulating Cells (LCCRH) in the Department of Pathology and Onco-Biology. In this unique platform LCCRH, they isolate, detect and characterize circulating tumor cells using combinations of the EPISPOT assay, the CellSearch® system (Silicon Biosystem - Menarini), the flow cytometry, the CellCollector (GILUPI), the molecular biology (AmpliSpeed device), the Parsortix system (Angle) and the DEPArray (Silicon Biosystem - Menarini) for single cell sorting. She has authored or co-authored >60 scientific publications in this field during the last years including 10 book chapters, she is the inventor of three patents in the liquid biopsy field and she is part of French national projects: for ex, PANTHER (FUI project) as well as of big European projects: CTC-SCAN (Transcan project), CANCER-ID (IMI project) and European Liquid Biopsy Academy (ELBA, Marie-Curie project).After she got the Scientific Prize given by the Region Languedoc-Roussillon in 2008, it was a great honor for her to receive the Gallet et Breton Cancer Prize, the highest honor conferred by the French Academy of Medicine in November 2012 and, very recently, the 2017 AACR Award for the most cited scientific article in 2015 (Cayrefourcq et al. Cancer Res).

T5: Functionnal Analyses of Circulating Tumor Cells in Cancer Patients

Catherine Alix-Panabières

Circulating tumor cells (CTCs) in blood are promising new biomarkers potentially useful for prognostic prediction and monitoring of therapies in patients with solid tumors including colon cancer. Moreover, CTC research opens a new avenue for understanding the biology of metastasis in cancer patients. However, an in-depth investigation of CTCs is hampered by the very low number of these cells, especially in the blood of colorectal cancer patients. Thus, the establishment of cell cultures and permanent cell lines from CTCs has become the most challenging task over the past year.

We described, for the first time, the establishment of cell cultures and a permanent cell line from CTCs of one colon cancer patient (Cayrefourcq *et al.* Cancer Res. 2015). The cell line designated CTC-MCC-41 is in culture for more than three years and has been characterized at the genome, transcriptome, proteome and secretome levels. This thorough analysis showed that CTC-MCC-41 cells resemble characteristics of the original tumor cells in the colon cancer patient and display a stable phenotype characterized by an intermediate epithelial/mesenchymal phenotype, stem-cell like properties and an osteomimetic signature indicating a bone marrow origin. Functional studies showed that CTC-MCC-41 cells induced rapidly *in vitro* endothelial cell tube formation and *in vivo* tumors after xenografting in immunodeficient mice.

More recently, we defined the molecular portrait of these metastasis-competent CTCs (Alix-Panabières *et al*. Clin Chem. 2017). These results highlight that CTC-MCC-41 line display a very specific transcription program completely different than those of the primary and metastatic colon cancer cell lines. Interestingly, among the 1,624 transcripts exclusively upregulated in CTC-MCC-41 samples, key genes related to energy metabolism, DNA repair and stemness genes were observed. Such data may supply insights for the discovery of new biomarkers to identify the most aggressive CTC sub-populations and for the development of new drugs to inhibit metastasis-initiator CTCs in colon cancer.

Moreover, the development of new immunotherapeutic strategies is of utmost importance. Antibodies against proteins that block the immune response of T-cells such as PDL1 have been approved for treatment of cancer patients after showing remarkable long-term remissions in subsets of patients. It is now important to develop predictive biomarkers to identify patients with the highest benefit from these therapies. In 2015, we could show for the first time that PD-L1 is heterogeneously expressed on CTCs from metastatic breast cancer patients (Mazel *et al.* Mol Oncol 2015). Further functional analysis of this interesting subset of CTCs might reveal special immunosuppressive properties.

Lukáš Valihrach

Institute of Biotechnology CAS, v. v. i., Czech Republic
http://www.ibt.cas.cz/

Lukas Valihrach is a postdoctoral fellow at Institute of Biotechnology CAS in BIOCEV, Vestec, Czech Republic. He has a broad experience with gene expression profiling using RT-qPCR and other techniques in different biological systems. Last 5 years his research is primarily focused on the single cell analysis in the field of glial cell biology (ischemic brain injury, neurodegenerative diseases). He is also active in the development of new methods (Two-tailed PCR for miRNA profiling) and quality control tools for single cell expression profiling.

T6: Single cell analysis of oligodendroglial lineage cells during post-ischemic regeneration

Valihrach Lukas [1], Valny Martin [2,3], Honsa Pavel [2], Matuskova Hana [2,3], Waloschkova Eliska [2], Kirdajova Denisa [2,3], Kriska Jan [2,3], Androvic Peter [1,4], Anderova Miroslava [2,3], Kubista Mikael [1,5]

[1] *Laboratory of Gene Expression, Institute of Biotechnology CAS, BIOCEV, Vestec, Czech Republic*

[2] *Department of Cellular Neurophysiology, Institute of Experimental Medicine CAS, Prague, Czech Republic*

[3] *2nd Faculty of Medicine, Charles University, Prague, Czech Republic*

[4] *Faculty of Science, Palacký University, Olomouc, Czech Republic*

[5] *TATAA Biocenter, Gothenburg, Sweden*

NG2 cells, a fourth glial cell type in the adult mammalian central nervous system, produce oligodendrocytes in the healthy nervous tissue, and display large differentiation potential under pathological conditions. However, their differentiation capacity is still poorly described in the context of focal cerebral ischemia (FCI).

To study the effect of FCI we used transgenic mice, which enables genetic fate-mapping of Cspg4-positive cells and their progeny, based on the expression of red fluorescent protein tdTomato. Differentiation potential of tdTomato-positive cells from control and post-ischemic brains was determined using the single cell RT-qPCR and immunohistochemistry. To analyze the changes of expression patterns caused by FCI we utilized self-organizing Kohonen maps, enabling us to divide NG2 cells and oligodendrocytes into subpopulations based on similarities of expression profiles of individual cells. We identified three subpopulations of NG2 cells emerging after FCI: proliferative; astrocyte-like and oligodendrocyte-like NG2 cells, phenotypes which were further confirmed by immunohistochemistry. We also utilized EdU labeling to disclose, that NG2 cells can differentiate directly into reactive astrocytes without proliferation. The difference between astrocyte-like NG2 cells and classical reactive astrocytes was determined using single cell RNA-Seq. Oligodendrocytes themselves formed four subpopulations, which reflected the process of oligodendrocytes maturation.

Taken together we identified several yet unknown differences between the expression profiles of NG2 cells and oligodendrocytes, and characterized specific genes contributing to phenotypical changes of NG2 cells after FCI and oligodendrocyte maturation. Moreover, our results pointed to the ability of NG2 cells to acquire multipotent phenotype after FCI, which was documented by generation of reactive astrocytes.

Evi Lianidou

Department of Chemistry, University of Athens, Greece
http://en.actc-lab.chem.uoa.gr/

Dr. Evi Lianidou is Professor of Analytical Chemistry and Clinical Chemistry at the Department of Chemistry, University of Athens, Greece. Dr Lianidou has established a Molecular Diagnostics Laboratory focused on Liquid Biopsy at the Department of Chemistry since 1998 (http://en.actc-lab.chem.uoa.gr/). Her lab is specializing in the Analysis of Circulating Tumor Cells (ACTC), and has access to many patient samples through extensive clinical collaborations. Her main research interests are on the development and clinical evaluation of: a) single-plex and multiplex quantitative RT-qPCR assays for the detection and molecular characterization of CTCs, b) multiplex assays for gene expression in CTCs based on the liquid bead array, c) DNA methylation assays in CTCs and ctDNA, d) highly sensitive assays for mutation analysis in CTCs and in ctDNA, and evaluation of circulating miRNAs as tumor biomarkers in plasma. Dr. Lianidou has 108 publications (https://www.ncbi.nlm.nih.gov/pubmed/?term=lianidou) and has organized together with Prof K. Pantel: a) the 7th International Symposium on Minimal Residual Disease in Athens, (http://ismrc2009.chem.uoa.gr), b) a scientific meeting on CTCs "Advances in Circulating Tumor Cells: From Basic Research to Clinical Practice" (www.actc2012.org), and c) the 2nd ACTC meeting, (October 8th-11th, 2014), in Crete, Greece (www.actc2014.org). Prof Lianidou is PI in the European TRANSCAN group "CTC-SCAN" and in the EU IMI Network Project "CANCER-ID" (www.cancer-id.eu) and serves on the Editorial Boards of many international journals including Clin Chemistry, Clin. Cancer Res, Breast Cancer Res, Cancer Res, Oncotarget, and many others. Dr. Lianidou is an elected member and Chair of the Committee for Clinical Molecular Biology Curriculum of the International Federation of Clinical Chemistry (IFCC), (http://www.ifcc.org/ifcc-education-division/emd-committees/c-cmbc/) and is coordinating the M.Sc. program of Clinical Chemistry, at the Department of Chemistry, University of Athens (http://en.clinical-chemistry.chem.uoa.gr/).

T7: CTC molecular characterization: potential and clinical applications

Evi Lianidou

Liquid biopsy provides a valuable source of biomarkers through simple and minimally invasive serial blood draws and represents a highly dynamic diagnostic, prognostic and theranostic tool for the management of cancer patients. Circulating tumor cells (CTCs) are major players in liquid biopsy and their presence has been linked to worse prognosis and early relapse in numerous clinical studies. CTC enumeration and molecular characterization offers an exciting approach to monitor the efficacy of systemic therapies in real-time, unravel the biology of cancer cell dissemination, understand resistance to established therapies and identify gene targets and signaling pathways relevant to therapeutic interventions. Single-cell CTC analysis is a powerful tool to understand tumor heterogeneity and the mechanisms involved in cancer progression with potential implications for improving treatment strategies. This overview is focused on the latest developments in the detection and molecular characterization of CTCs, and their clinical applications in many types of cancer.

Leon WMM Terstappen

University of Twente, Enschede, Netherlands
https://www.utwente.nl/

Professor Leon WMM Terstappen, MD, PhD is chair of the department of Medical Cell BioPhysics at the University of Twente, The Netherlands. He received his medical degree from the University of Groningen in 1983 and his PhD in 1988 from the University of Twente. In 1987 he started as post-doctoral fellow at the research department of Becton Dickinson Immunocytometry systems in Mountain View CA, USA and held various research positions thereafter. In 1994 he started as Chief Scientific Officer at Immunicon Corporation, Huntingdon Valley, PA, USA and in 2007 he was appointed at the University of Twente. He is an international recognized expert in cytometry and pioneered the detection of rare cells. He was in charge of the development of the CellSearch system, the only FDA cleared platform to enumerate circulating cancer cells in blood, which was awarded the "Prix Galien", the major prize for in-vitro diagnostics devices. He currently is the program leader of the STW Perspectief program Cancer-ID and co-leader of the EU IMI program CANCER-ID both programs aim to develop and validate "liquid biopsies" technologies for personalized cancer care. Terstappen is a recipient of the Max Fulwyler Award for Innovative Excellence.

T8: Tools to identify and characterize single Circulating Tumor Cells

Leon WMM Terstappen

Circulating tumor cells (CTC) are cancer cells disseminated into the blood from primary or metastatic sites. The presence of CTC is predictive of relatively short survival in metastatic carcinomas, and the more CTC are present the worse the outcome. The promise of CTC as a real time liquid biopsy to guide therapy can only be met when CTC are representative of the tumor, present in the blood volume analyzed, can be detected, isolated and therapy relevant information extracted. Heterogeneity of the tumor cells moreover demands analysis at the single cell level to assess this heterogeneity and determine its implications for a therapy to be effective. Preferably all treatment relevant molecular pathways need to be identified at the protein, RNA and DNA level. To realize this advance in technologies are needed and several technologies will need to be combined to realize a liquid biopsy to guide therapy of cancer patients. Here we show the tools under development for the selection of single cancer cells and probing their content for mRNA and DNA and measuring the products secreted by the single cells.

Nikolas Stoecklein

University Hospital of the Heinrich-Heine-University, Germany
http://www.uniklinik-duesseldorf.de/

Nikolas Stoecklein is currently a Professor for Experimental Surgical Oncology at the Heinrich-Heine University Düsseldorf (HHU), Germany. His major research interests are minimal residual disease and early systemic disease in cancer. A current focus of his group is to improve CTC-based liquid biopsies. He pioneered Diagnostic Leukapheresis (DLA) for enhanced CTC detection and established workflows to analyze the rare CTCs comprehensively at single cell level. Prior joining the HHU, Nikolas obtained post-doctoral training at the Institute of Immunology of the Ludwig-Maximilians-University Munich, Germany, in the group of Christoph Klein. He graduated from the University of Hamburg, Germany, in 1998 where he studied medicine.

T9: Molecular Characterization of CTCs detected with the CellSearch System

Nikolas Stoecklein

Circulating tumor cells (CTCs) enumerated with the CELLSEARCH system (CS) have strong prognostic significance in several cancer types and might have clinical utility for prediction. However, the far greater potential lies in the molecular characterization of CTCs to gain direct insights into the biology of systemic cancer, but also to use the information for treatment decisions. Yet, CTC isolation and their comprehensive analysis remain technically quite challenging. We therefore developed robust workflows to isolate and analyze single CTCs for genetic and epigenetic alterations. This allowed us, for example, to monitor the genomic profiles of CTCs during therapy, revealing strong clonal relationships, but indicated also selection of certain genotypes surviving systemic therapy. Such selected alterations might help to identify genetic resistance mechanisms. A major challenge to use CTC-based liquid biopsies is the extreme low concentration of CTCs and the minimal amount of investigated blood in standard CTC-tests. To tackle this problem, we introduced the pre-enrichment step Diagnostic Leukapheresis (DLA). DLA allows to screen liters of blood and significantly increases CTC yield, enabling "real" liquid biopsies.

T10: Character of circulating tumor cells as a part of liquid dynamic medicine in oncology

K. Kolostova[1], E. Pospisilova[1], J. Spicka[1], P. Eliasová[1], Z. Bielcikova[2], L. Krizova[2], M. Vocka[2], I. Kiss[1,3], V. Maly[4], M. Cegan[4], V Bobek [1,4,5,6]

[1] *Department of Laboratory Genetics, University Hospital Kralovske Vinohrady, Prague, Czech Republic*

[2] *Department of Oncology, First Faculty of medicine Charles University and General University Hospital in Prague, Czech Republic*

[3] *Department of Gynaecology, Military Hostpital, Prague, Czech Republic*

[4] *Department of Thoracic Surgery Masaryk´s Hospital, Krajska zdravotni a.s Usti Nad Labem, Czech Republic*

[5] *Department of Histology and Embryology, Wroclaw Medical University, wybrzeże Ludwika Pasteura 1, 50-367 Wrocław, Poland*

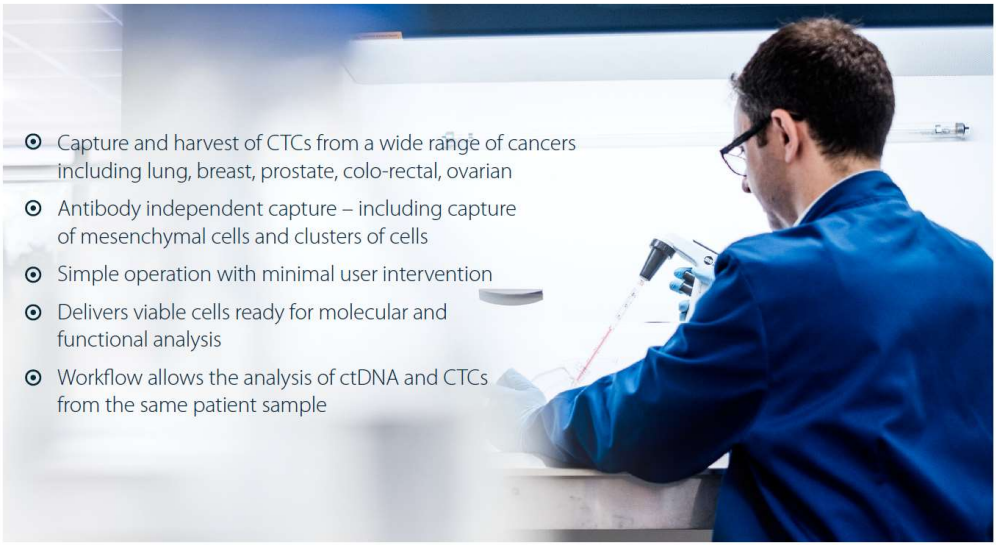
[6] *3rd Department of Surgery University Hospital Motol and 1st Faculty of Medicine Charles University, Prague*

The growing application of liquid biopsy to dynamically track tumor disease and to adapt anticancer therapy according to real – time data on cancer burden is shaping modern medical oncology. The liquid biopsy testing can be provided for a cancer patient in a non-invasive manner by simple blood withdrawal including circulating tumor cells (CTC) and/or free circulating tumor DNA (ctDNA) analysis.

In order to better describe new therapeutic paradigm the term "Liquid dynamic medicine" we conducted CTC- testing as a part of daily laboratory routine for up to 2000 patients in 2017-2018 with diagnosed solid tumors (breast cancer n=620, lung cancer n=460, GIT-tumors n= 440). We inteded to characterize several of the patients in so called N-of-1 trial where each patient acts as tester and control of aplied therapy. The serial CTC-analysis included cytomorphological evulation of the viable size-based enriched CTCs (CTC-number) and gene expression analysis of the tumor-associated genes and genes associated with chemoresitance. Newly, next generation sequencing (NGS)– plaform Genereader (Qiagen) has been implemented to provide standardized quality of automatized CTC- NGS-data analysis by QCI-Analyze and QCI- Interpret software. QIAact Actionable Clinical Insight Tumor Panel (Qiagen) covering 12 mostly mutated genes in solid tumors has been used targeting on mutations in 773 regions. Implementation of CTC- navigated therapy into the clinic could aid personalized treatment and permit access to a broader armamentarium of cytostatics for patients when indicated.

Capture and harvest of Circulating Tumor Cells from blood*

ANGLE's Parsortix™ technology captures circulating tumor cells (CTCs) from blood*. The resulting "liquid biopsy" enables the genetic and protein analysis of the patient's cancer which has the potential to help direct optimal therapy – so moving towards personalised cancer care.



- Capture and harvest of CTCs from a wide range of cancers including lung, breast, prostate, colo-rectal, ovarian
- Antibody independent capture – including capture of mesenchymal cells and clusters of cells
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- Delivers viable cells ready for molecular and functional analysis
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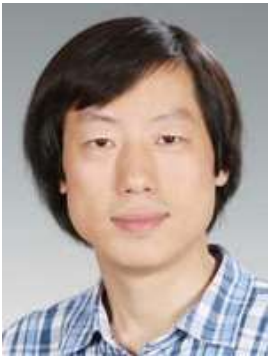
For more information and latest publications please visit www.angleplc.com or email parsortix@angleplc.com

ANGLE

Fuchou Tang



Biodynamic Optical Imaging Center, Integrated Science Research Center, Peking University, China
http://biopic.pku.edu.cn/

Dr. Fuchou Tang is Professor at BIOPIC, College of Life Sciences, Peking University. He is also Associate Director of Beijing Advanced Innovation Center for Genomics. He set up his own lab as a principal investigator at Peking University in 2010. Dr. Fuchou Tang's lab focuses on the epigenetic regulation of gene expression network during human early embryonic development and germline development (Cell, 2013; Nature, 2014; Cell Stem Cell, 2014; Cell, 2015; Science, 2015; Nature, 2016; Cell Stem Cell, 2017a, 2017b, 2017c). His lab pioneered the single cell sequencing field and has systematically developed a serial of single cell functional genomic sequencing technologies [scRRBS (Genome Research, 2013); scMicrofluidic-seq (PNAS, 2014); SUPER-seq (Genome Biology, 2015); scTrio-seq (Cell Research, 2016); scCLEVER-seq (Cell Stem Cell, 2017b); scCOOL-seq (Cell Research, 2017); MR-seq (Science Bulletin, 2017)]. His work has been cited for more than 5,000 times. Some of his work has been selected as Top 10 scientific and technological progresses of China in the year of 2014 and Top 10 scientific and technological progresses of China in the year of 2015. He is editorial board members of Science Bulletin, Genome Biology, & Open Biology. He organized the Cold Spring Harbor Asia conference of Frontiers in Single Cell Genomics in 2016.

T11: Decoding the gene regulation network in human germline cells using single-cell functional genomics approaches

Fuchou Tang

Human germline cells are crucial for maintenance of the species. However, the developmental trajectories and heterogeneity of human germline cells remain largely unknown. We performed single-cell RNA-seq and DNA methylome sequencing analyses of human germline cells in female and male human embryos spanning several critical developmental stages. We found that female fetal germ cells (FGCs) undergo four distinct sequential phases characterized by mitosis, retinoic acid signaling, meiotic prophase, and oogenesis. Male FGCs develop through stages of migration, mitosis, and cell-cycle arrest. Individual embryos of both sexes simultaneously contain several subpopulations, highlighting the asynchronous and heterogeneous nature of FGC development. Moreover, we observed reciprocal signaling interactions between FGCs and their gonadal niche cells, including activation of the bone morphogenic protein (BMP) and Notch signaling pathways. Our work provides key insights into the crucial features of human germline cells during their highly ordered mitotic, meiotic, and gametogenetic processes *in vivo*.

Norman Dovichi

University of Notre Dame, USA
http://chemistry.nd.edu/

Norman Dovichi is the Grace-Rupley Professor of Chemistry and Biochemistry at the University of Notre Dame. In the 1980s, he introduced the concept of single molecule detection to the chemical literature. In the 1990s, his group developed capillary array electrophoresis instruments for high-throughput DNA sequencing. He was recognized for this work by the journal Science as an "Unsung Hero of the Human Genome Project". Over the last decade, his group has focused its attention on coupling capillary electrophoresis with tandem mass spectrometry as a tool for high throughput and high sensitivity proteomics. Recent results include detection of 10,000 peptides in an 80-min single-shot separation of the HeLa proteome, detection of 2,300 phosphopeptides in a 100-min single shot separation, and detection of low zeptomole amounts of tryptic peptides in complex mixtures. Finally, his group used HPLC-MS/MS for the first proteomic analysis of vertebrate development. Over 5,500 proteins were quantified at six stages of early development of Xenopus laevis. This work has been extended to the characterization of protein expression in single blastomeres isolated from embryos at the 2, 4, 8, 16, 32, and 50-cell stage. These are the first comprehensive studies of protein expression in single vertebrate cells.

T12: Proteomics of early development: the assembly of the vertebrate brain

Norman Dovichi

Abstract: *Xenopus laevis* is an important model organism for early vertebrate development. Fate maps have been developed for cells within the early stage embryo.The major progenitors of the central nervous system, including the brain and retina, come from the D1 blastomere, which is found in the 8-cell embryo. We have injected the D1 blastomere with fluorescent tracer, and allowed the embryo to develop further. We then microdissected fluorescent material from embryos at the 8, 16, 32, blastula, gastrula, neurula, and late neurula stages of development. This material was subjected to both proteomic and transcriptomic analysis. Nearly 6,000 proteins and over 45,000 transcripts were quantified across the development stages. Merging of these large datasets is challenging, and our latest results will be presented at the conference.

Radek Šindelka

Institute of Biotechnology CAS, v. v. i., Czech Republic
http://www.ibt.cas.cz/

Radek Šindelka is working as an independent scientist at the Institute of Biotechnology of the Czech Academy of Sciences. He uses African-clawed frog embryos as a model for asymmetric localization during early development and for identification of molecular regulation of wound healing and regeneration.

T13: Subcellular transcriptome and proteome analyses to reveal body plan determinants of early development

Radek Šindelka

Complex bodies of organisms develop after fusion of two cells: sperm and egg and their plans are defined by formation of body axes such as left-right, anterior-posterior (head-toe) and dorsal-ventral (back-belly). Body axes are usually determined by asymmetrical localization of biomolecules in gradients and following asymmetrical cell divisions. We use combination of biological models (*Xenopus laevis* eggs and early embryos) and high-throughput approaches (RT-qPCR, RNA-seq and iTraq UPLC-ESI-MS/MS) to identify asymmetrically localized determinants among coding and noncoding RNAs and proteins.

T14: SMARTer Solutions for Single-Cell Transcriptomics and Genomics (Takara)

Samuel Bouvet[1]

[1] *Takara Bio Europe*

Since the emergence of Next-Generation Sequencing (NGS), the importance and demand for single-cell analysis have risen rapidly. As a result, single-cell RNA-Seq and single-cell DNA-seq have been gaining prominence not only in basic research fields, but also in clinical applications.

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Vessela N. Kristensen

Institute of Clinical Medicine, Department of Clinical Molecular Biology, Norway
https://www.med.uio.no/

Vessela N. Kristensen is a Professor I at the Medical Faculty of the University in Oslo (UiO) in Clinical Epidemiology at the Department of Clinical Molecular Biology and Lab science (EpiGen), Akershus university hospital, and a Group Leader at the Department of Genetics, IKF, Det Norske Radiumhospital and Department of Clinical Molecular Biology at EpiGen, Ahus. She has been also a visiting professor at Princeton University (2012), Professor II at the Centre for Integrative Genetics, University of Life Sciences, Ås (2004-2006) and assistant professor at the Advanced Technology Center at NCI, NIH, Bethesda (2003). In 1995 Kristensen was a postdoc at the Berzelius Laboratory at Karolinska in the group of professor Magnus Ingelman Sundberg. She was also granted a fellowship at Fujita Health University, Nagoya, Japan in 1999, with professor Nobuhiro Harada.
Vessela Kristensen graduated the Faculty of Natural Sciences, Charles University, Prague in 1990 and got her PhD from the Faculty of Medicine at the same university in 1996. The same year she moved to Norway. In the course of her 20 year career in Norway Kristensen has published 218 peer reviewed research papers with 6137 citations, brought up 16 PhD students to successful thesis defence as a main supervisor, and authored and co-authored around 20 scientific grants. Her career as a group leader started in 2003 with a career development research grant from the Research Council of Norway "Integral approach to characterize clinically and therapeutically relevant biological pathways in human cancer". This field is a main topic of her research also today. Her h index is 44 and her Research Gate score is 47.71.

T15: Tracing the origin of disseminated tumor cells in breast cancer using single-cell sequencing

Vessela N. Kristensen

BACKGROUND: Single-cell micro-metastases of solid tumors often occur in the bone marrow. These disseminated tumor cells (DTCs) may resist therapy and lay dormant or progress to cause overt bone and visceral metastases. The molecular nature of DTCs remains elusive, as well as when and from where in the tumor they originate. Here, we apply single-cell sequencing to identify and trace the origin of DTCs in breast cancer.
RESULTS: We sequence the genomes of 63 single cells isolated from six non-metastatic breast cancer patients. By comparing the cells' DNA copy number aberration (CNA) landscapes with those of the primary tumors and lymph node metastasis, we establish that 53% of the single cells morphologically classified as tumor cells are DTCs disseminating from the observed tumor. The remaining cells represent either non-aberrant "normal" cells or "aberrant cells of unknown origin" that have CNA landscapes discordant from the tumor. Further analyses suggest that the prevalence of aberrant cells of unknown origin is age-dependent and that at least a subset is hematopoietic in origin. Evolutionary reconstruction analysis of bulk tumor and DTC genomes enables ordering of CNA events in molecular pseudo-time and traced the origin of the DTCs to either the main tumor clone, primary tumor subclones, or subclones in an axillary lymph node metastasis.
CONCLUSIONS: Single-cell sequencing of bone marrow epithelial-like cells, in parallel with intra-tumor genetic heterogeneity profiling from bulk DNA, is a powerful approach to identify and study DTCs, yielding insight into metastatic processes. A heterogeneous population of CNA-positive cells is present in the bone marrow of non-metastatic breast cancer patients, only part of which are derived from the observed tumor lineages.

Reference: Genome Biol. 2016 Dec 9;17(1):250.
Highlighted in Science TM http://stm.sciencemag.org/content/9/371/eaal4988

T16: Molecular analysis of single circulation tumor cells from cancer patients

Pinzani Pamela [1], **Salvianti Francesca** [1]

[1] *University of Florence, Dept. of Clinical, Experimental Biomedical Sciences*

Introduction: Circulating tumor cells (CTCs) are a real-time "liquid biopsy" of the tumor reflecting the disease complexity at any stage of cancer progression. Single-cell analysis may help to elucidate tumor heterogeneity at the CTC level. Technical advances have enabled molecular analyses at the single-cell level allowing the profiling of rare cancer cells in clinical samples but this is a multi-step process that remains technically challenging.
Aims: Our studies aimed at exploring the feasibility of single-cell mutational analysis and gene expression profiling on circulating tumor cells from cancer patients.
Materials and Methods: CTCs were enriched from the blood of cancer patients and recovered singularly or in pools by the single-cell sorter DEPArray. The mutational analysis of CTCs was performed by Sanger or NGS upon whole genome amplification. Gene expression analysis of multiple genes was performed by RT-qPCR after cell lysis, reverse transcription and preamplification of cDNA.
Results: We successfully sequenced by Sanger as well as NGS CTCs from cancer patients at the single-cell level revealing intra-patient heterogeneity in the mutational status of single CTCs. We demonstrated the feasibility of multiple gene expression analysis in pools of few CTCs from cancer patients after optimization of the preanalytical procedures.
Conclusion: We contributed to the demonstration of the feasibility of the molecular characterization of single circulating tumor cells from cancer patients. Single CTC analysis is a promising tool for future clinical applications focused on non-invasive disease monitoring. Several steps in the way of standardization of the pre-analytical, analytical and post-analytical procedures must be undertaken to introduce single CTC mutational analysis into clinical practice.

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Liquid Biopsy: Cells for Precision Medicine

Mikael Kubista

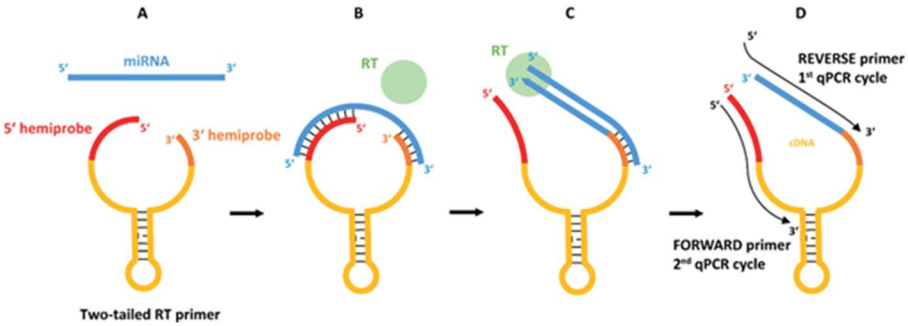
Institute of Biotechnology CAS, v. v. i., Czech Republic
http://www.ibt.cas.cz/

Dr Kubista is founder and CEO of the TATAA Biocenter and the head of the department of gene expression at the Institute of Biotechnology of the Czech Academy of Sciences. He was one of the pioneers contributing to the development of quantitative real-time PCR (qPCR) and introduced qPCR for single cell expression profiling. He led the development of reagents for high throughput expression profiling and quality control at TATAA Biocenter and he developed qPCR tomography for intracellular expression profiling.

T17: Two Tailed-PCR for precision diagnostics

M. Kubista,.P. Androvic, L. Valihrach, J. Elling, R. Sjöback

We present a highly specific, sensitive and cost-effective system to quantify miRNA expression based on novel chemistry called Two-tailed PCR. Two-tailed PCR takes advantage of target-specific primers for reverse transcription composed of two hemiprobes complementary to two different parts of the targeted miRNA, connected by a hairpin structure. The introduction of a second probe ensures high sensitivity and enables discrimination of highly homologous miRNAs irrespectively of the position of the mismatched nucleotide. Two-tailed RT-qPCR has a dynamic range of 7 logs and a sensitivity sufficient to detect less than ten target miRNA molecules. The reverse transcription step can be multiplexed and it allows for rapid testing with a total analysis time of less than 2.5 hours.



T18: Screening circulating tumor cells as a non-invasive cancer test in 3,388 individuals from high-risk groups (ICELLATE2)

Christer Ericsson, Juan Castro, Luis Sanchez, María Teresa Nuñez, Ming Lu Tomas Castro, Hamid R. Sharifi

Cancer is known to spread up to 12 years before clinical symptoms occur, but few screening tests exist. Early detection would give the opportunity for early treatment, potentially improving prognosis. To this end 3,388 subjectively healthy individuals of age 45 to 80 who had been exposed to cancer risk factors were screened for the occurrence of circulating tumor cells in their blood. Presence of circulating tumor cells is a suspicious finding indicative of spreading cancer, since cancer metastasizes by way of the blood, and offers the opportunities to a) follow up the individual clinically based on established guidelines for early detection of cancer and b) evaluate the cells further analytically. 107 individuals showed one or more circulating tumor cells in a 7.5 ml blood sample, which constitutes a positive circulating tumor cell test, based on the iCellate IsoPicTM laboratory test. That number compares favorably with the cancer incidence per 100,000 people/year that is 157.1 in Peru, given that a high-risk group of individuals was screened and that the screening results would be expected to correspond to an accumulated incidence of up to 12 years. The present findings therefore identify screening for circulating tumor cells as a promising new test.

T19: cellenONE X1 : A versatile platform for single cell isolation (Cellenion)

Guilhem Tourniaire

cellenONE® X1 technology offers unmatched accuracy in single cell isolation thanks to its technological fundamentals: working as a picovolume liquid handler able to isolate a single cell in a liquid drop and dispense it with micron precision in any labware. The success of our patented technology relies on a deterministic approach and real-time feedback to our users, hence offering the precision and confidence required by the growing single-cell sequencing and cloning markets.
Next generation sequencing is revolutionizing our approach to medicine. By interrogating individual tumor cells, it is now possible to better understand cancer evolution and resistance. cellenONE plays a major role in cancer research as it allows isolation of single tumor cells and also single nuclei from a variety of sample type (fresh, frozen, formalin fixed). It also offers a new paradigm for rare samples as it allow up to 95% recovery from samples containing as little as 100 cells making it ideal for clinical sample processing. Finally, cellenONE is an open platform letting users choose their preferred consumables while allowing a wide range of downstream analyses (scRNA-Seq, WGA, scATAC-Seq…).

Pascal Barbry

Université Côte d'Azur, Institut de Pharmacologie Moléculaire et Cellulaire, France
http://www.ipmc.cnrs.fr

Pascal Barbry's early work focused on identifying the structure of ion channels involved in epithelial ion transport in tissues. This initial work develop approaches and tools to study several ion channels (epithelial Na+ channel, CFTR, etc). It enabled the characterization of mutant forms of CFTR which are responsible for cystic fibrosis, and to the identification of the first pharmacological molecule able to activate them. This discovery was one of the earliest evidence that a pharmacological approach could be beneficial to the patients. His current work focuses on RNA, including non-coding RNAs, which do not encode any protein, but may have significant biological effects. Pascal Barbry studies them in the context of pathologies such as cystic fibrosis, asthma, pulmonary fibrosis, lung cancers. Some microRNAs, for instance, are important during differentiation of airway epithelial cells. Using large-scale approaches, the researcher and his team at IPMC (Sophia Antipolis, France) is now trying to reconstruct the successive events that contribute to the differentiation of an airway epithelial cell, and how a group of neighbouring cells develop a sort of collaborative ecosystem, where distinct cells can fulfill complementary tasks. RNA sequencing, including at the single cell level, genomics and bioinformatics techniques are instrumental to perform this research that Pascal Barbry and his team combine with molecular and cellular approaches.

T20: A new functional genomics approach to understand the human airways at a single cell level

Pascal Barbry

Mammalian airways are lined by a pseudo-stratified mucociliary epithelium that constitutes a first line of defense of the respiratory tract through mucociliary clearance. This epithelium forms a complex ecosystem made of different cell types. Understanding the fate and organization of the different cell types can help to develop new treatments against severe human respiratory diseases. Our approach is to identify by single cell RNA-seq the airway cell composition along the human respiratory tree in healthy subjects. We have set up protocols to collect by bronchoscopies and then analyze the different cell types located in the airways by the 10X Chromium technology. The single cell isolation protocol, made from biopsies (10-20 mg) or brushings (100-200,000 cells), does not affect cell viability and allows the generation of robust gene expression profiles. A comprehensive cell atlas of human airways is currently being established, based on samples derived from nose, trachea, and different branches of bronchi. Our bioinformatic pipeline take into account several peculiarities of droplet based scRNAseq studies: presence of a background noise or low RNA content cell types deriving from 'empty droplets', assessment of good quality cells and outliers, the identification of doublet cells, PCA dimension reduction and clustering, differential analysis of the different cell types that are observed. First results already identify specific populations of cells, such as deuterosomal cells, which correspond to precursor of multiciliated cells [Revinski, Nature Communications, In Press], or ionocytes, which express high levels of ion transporters, such as CFTR, the cystic fibrosis gene product. By using bronchoscopy approaches, which are routinely used in pneumology, we anticipate a fast adoption of these novel approaches by the clinic, in order to tacke more efficiently airway diseases.

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T21: Single cell Viral Copy Number analysis using ddPCR

Santeramo Ilaria [1], Di Cerbo Vincenzo [1], Hassan Enas [1], Thwaites Tristan [1], Surmacz-Cordle Beata [1], Marshall Damian [1]

[1] *Cell and Gene Therapy Catapult, London, UK*

Introduction: Gene modified cell therapies are emerging as a promising approach to the treatment of complex diseases. These therapies are based on the transduction of the gene of interest into the target cells using viral vectors. Among the analytical specifications of a genetically modified product, the analysis of the Viral Copy Number (VCN) is included as it informs on the safety profile of the product and the consistency of the transduction processes. However, the VCN is commonly measured on the bulk population, which does not provide information on cell-cell variation. Aims This work aims to reliably quantify the VCN in single cells and investigate cell-to-cell variability in gene therapy products.

Materials and Methods: Purified T cells from a healthy donor were transduced with an exemplar lentiviral (LV) vector expressing ZsGreen. Following cell sorting, the cell suspension is loaded into the C1 single cell auto prep system (Fluidigm) that captures and processes up to 96 cells in nanolitre-sized chambers to facilitate the pre-amplification of genetic material. The harvested material is then subjected to droplet digital PCR (BioRad) using LV-specific Taqman assays together with a copy number reference gene. An in-house statistical framework is then applied to obtain an estimation of the VCN from each cell.

Results and Conclusion: Our results show that (i) viral copy number at a single cell level is consistent with the bulk-VCN, thereby supporting the precision and robustness of our method; (ii) the VCN heterogeneity at single cell level can be monitored with a simple and largely automated assay. Importantly, this method offers the advantage of a full customization of the analysed transgenes and therefore may be easily adapted for different gene delivery systems. We believe that the application of this method may help monitoring the production of cell therapy products and overall contribute to their safe and efficacious delivery to patients.



Single Cell Analysis

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T22: SureCell ATAC-Seq: A new platform for Single Cell ATAC-seq using Bio-Rad's droplet digital technology

Jennifer Chew[1], Fabiana Duarte[2], Caleb Lareau[2], Zachary Burkett[1], Vinay Kartha[2], Andrew Kohlway[1], Jason Buenrostro[2], Ronald Lebofsky[1]

[1]*Bio-Rad Laboratories, Digital Biology Center*

[2]*Broad Institute, MIT-Harvard*

Cellular states and the activation of gene expression are governed by functional interactions between transcription factors and DNA regulatory elements. scATAC-seq (Single Cell Assay for Transposase Accessible Chromatin using sequencing) is a powerful tool to interrogate chromatin accessibility genome-wide at single-cell resolution. To enable the widespread and facile application of this approach, we developed a scATAC-seq platform using Bio-Rad's Droplet Digital technology. Here, we describe a simple workflow that enables processing of thousands of cells with improved sensitivity and signal-to-noise relative to previously published work. We apply this technology to profile the epigenomes of tens of thousands of single cells across normal hematopoiesis and in response to immunological stimuli to discover a large repertoire of lineage- and stimulus-specific non-coding regulatory elements and transcription factors. Altogether, we show that these rich data sets and experimental tools provide a new platform for discovering regulatory differences across cells within heterogeneous tissues, enabling the discovery of *cis*- and *trans*-acting effectors of cellular differentiation and disease.

Michael W. Pfaffl

Technische Universität München, Freising-Weihenstephan, Germany
https://www.tum.de/

Michael W. Pfaffl started 1986 to study 'Agriculture - Animal Science' and 'Biotechnology' at the Technical University of Munich (TUM). In 1997 he obtained his PhD in 'Molecular Physiology' in the field of molecular muscle and growth physiology at the Chair of Physiology. In June 2003 he completed his Venia Legendi (Dr. habil.) at the Center of Life and Food Sciences Weihenstephan with the title 'Livestock transcriptomics -- Quantitative mRNA analytics in molecular endocrinology and mammary gland physiology'.

Early 2010 he became Professor of 'Molecular Physiology' at the TUM School of Life Sciences. Today he has reached the 'Principal Investigator' status at the Institute of Animal Physiology & Immunology and is one of the leading scientists in the field of Gene Quantification, RT-qPCR technology, RNA sequencing, and complex data analysis in mRNA & small-RNA expression profiling.

He is author of around 180 peer reviewed publications, 45 book chapters, and held more than 230 lectures worldwide. In March 2012 the Elsevier SciVerse Scopus Award 2012 was granted to Prof. Michael W. Pfaffl, whose top cited Scopus article entitled "A new mathematical model for relative quantification in real-time RT-PCR" published 2001 in Nucleic Acids Research 29(9) has been cited today more than 21,800 times.

He is coauthor of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines (2009) and coauthor of the dMIQE guidelines for digital PCR (2013).

Professor Michael W. Pfaffl has editorial involvements as Founding- & Section-Editor in 'Biomolecular Detection and Quantification', Editor in 'Methods' and 'International Journal of Oncology', and Editor-in-Chief of the 'Gene Quantification' webportal (www.gene-quantification.info), the world biggest webpage around qPCR, dPCR and Gene Expression profiling techniques and applications. He is initiator and lead organizer of the qPCR, dPCR & NGS Gene Quantification Event series in Freising Weihenstephan in Germany since 2004 (www.eConferences.de).

T23: Exosomal microRNA Biomarkers Signatures in Clinical Diagnostics

Michael W. Pfaffl

Extracellular vesicles (EVs) are circulating in body liquids and are involve in the intercellular communication with various key functions in physiological and pathological processes. In recent time especially the exosomes have gained huge interest because of their molecular diagnostic potentials based on the containing microRNA signature. The past decade has brought about the development and commercialization of a multitude of extraction methods to isolate EVs and/or exosomes, with major focus on blood compartments. The exosome purity and which subpopulations of EVs are purified strongly depend on the applied isolation method, which in turn determines how suitable resulting samples are for potential downstream applications and biomarker discovery. Herein we compared the performance of various optimized isolation principles for serum EVs/exosomes in healthy individuals and critically ill patients. The isolation methods were benchmarked regarding their suitability for microRNA biomarker discovery as well as biological characteristics of captured vesicles. Isolated vesicles were deeply characterized by nano tracking analysis (amount, size, distribution), transmission electron microscopy (size, appearance), western blotting (surface marker proteins, total protein), small-RNA next generation sequencing (focus on microRNA families), and RT-qPCR (validation of microRNA biomarker signature). To analyze the high complex small-RNA sequencing results, a self-established bioinformatics pipeline for microRNA (based on R) and a deeper analysis of their isoforms (isomiRs) was developed and successfully applied (isomiRROR). Goal was the development of a 'microRNA/isomiR biomarker signature' for the early diagnosis and for a valid classification of sepsis patients. The results provides guidance for navigating the multitude of EV and exosome isolation methods available today, and helps researchers and clinicians in the field of molecular diagnostics to make the right choice about the EV and/or exosome isolation strategy in microRNA/isomiR biomarker signature development.

T24: Search for prognostic and predictive markers in sporadic CRC

Vodicka P[1,2,3], Vymetalkova V[1,2,3], Vodenkova S[1,2,4], Jiraskova K[1,2], Opattova A[1,2,3], Kroupa M[1,3], Vodickova L[1,2,3]

[1]*Institute of Experimental Medicine Czech Academy of Sciences, Prague, Czech Republic*

[2]*First Medical Faculty, Charles University Prague, Czech Republic*

[3]*Medical Faculty in Pilsen, Charles University Prague and Biomedical Center in Pilsen, Pilsen, Czech Republic*

[4]*Third Medical Faculty, Charles University Prague, Czech Republic*

State of the art: Cellular genomic stability is maintained by DNA repair and DNA damage response – impairment of DNA repair capacity (DRC) in malignant cells may implicate better therapeutic outcomes of oncological patients due to the accumulation of DNA damage and consequent apoptosis of malignant cells. Alternatively, the patients with tumor cells exhibiting high DRC may contend with poor response, resistance to treatment and decreased survival. We investigated DRC of excision repair in target tissue as a predictive marker for a treatment strategy and long-term survival in patients with newly diagnosed colon cancer. Additionally, we explored links between functional genetic polymorphisms (SNPs) in DNA repair genes covering the main DNA repair pathways, the risk of colorectal cancer (CRC) and clinical outcomes. Methodology: Our set of patients (with data on microsatellite stability and 5-FU treatment) was followed-up at least for 30 months. Tumor tissue and adjacent mucosa samples were obtained at surgical resection. Protein extracts from tissues were isolated both for protein expression (Western Blot) analysis and for measurement of DRC. Functional DRC was performed by comet assay-based *in vitro* DNA repair assay. Functional and genomic databases enabled identification of DNA repair gene variants with functional consequences. Findings: DNA repair gene variants were proven to modulate clinical outcome of colorectal cancer. In CRC patients, interestingly, the DNA repair capacity, significantly lower at the time of diagnosis, increased to the levels observed in healthy control subjects following the completion of chemotherapy. There are interesting associations between DRC measured in tumor tissue, adjacent mucosa and peripheral blood lymphocytes. Base excision repair capacity exerted only marginal effect on overall survival. Conclusions: Present results identify plausible candidate DNA repair gene variants affecting survival of CRC patients and clinical outcome of the disease. DRC may constitute predictive biomarker in colorectal cancer therapy and targeting DNA repair processes may pose clinical benefit in cancer treatment.

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Poster session

Single cell Applications

P1: Impact of blood preservation tubes and long-term storage of circulating tumor cells on performance of whole genome amplification

Anna Babayan [1], Malik Alawi [2], Melanie Janning [1,3], Volkmar Müller [4], Harriet Wikman [1], Maria Geffken [5], Sonja Loges [1,3], Simon A. Joosse [1], Klaus Pantel [1]

[1] *Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

[2] *Bioinformatics Core, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; Heinrich-Pette-Institute, Leibniz-Institute for Experimental Virology, Hamburg, Germany*

[3] *Department of Oncology, Hematology and Bone Marrow Transplantation with section Pneumology, Hubertus Wald University Comprehensive Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

[4] *Department of Gynecology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

[5] *Department of Transfusion Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

Background: Given the role of cancer heterogeneity, single cell analysis might help to decipher the ongoing cancer evolution and to observe the emergence of therapy-relevant genomic aberrations. In contrast to cell-free tumor DNA, analysis of individual circulating tumor cells (CTCs) allows for investigation of pure tumor DNA without wtDNA background. However, one single cell contains only 7-8pg DNA, making whole genome amplification (WGA) a first mandatory step for any downstream analysis. Despite different technologies for WGA are currently available, their effectiveness in combination with NGS, material preservation and long-term storage is currently unknown.

Materials and methods: Cell culture cells and CTCs were fixed, stained and individually picked by micromanipulation. Ampli1 and PicoPlex WGA kits were utilized as well as HiSeq2000 and IonTorrent NGS platforms. Performance of the WGA kits was evaluated in context of yield and quality of the amplified DNA, impact of the blood preservation method and utilization of FFPE material, short- and long-term storage of CTCs, and suitability of the WGA product for downstream NGS-based application.

Results: In total, more than 250 single cells were analyzed. No significant impact of blood collection tubes (EDTA, CellSave) or FFPE material was observed on the WGA efficacy. On average, Ampli1 outperformed PicoPlex in terms of DNA yield (6.8 µg vs 3.7 µg, respectively). In combination with NGS, the best statistics for the identification of SNPs and indels present in bulk reference DNA were observed for Ampli1-processed material as compared with PicoPlex within the same sequencing platform. Short-term stored (≤ 6 months) individual cell culture cells and CTCs can be amplified by Ampli1 with success rates of quality control (QC) of 95% and 76%, respectively. Individual long-term stored (≥ 5 years) CTCs passed the WGA-QC in 53% of cases.

Conclusion: Ampli1 WGA kit is suitable for single cell analysis of short-term and long-term stored CTCs.

P2: The role of the fusion oncogenes and cancer stem cells in myxoid liposarcoma

Soheila Dolatabadi [1], Emma Jonasson [1], Malin Lindén [1], Milagrosa López Riego [1], Christoffer Vannas [1], Lisa Andersson [1], David Švec [2], Pierre Åman [1], Anders Ståhlberg [1]

[1] *Sahlgrenska Cancer Center, Department of Pathology and Genetics, Institute of Biomedicine, Sahlgrenska Academy at University of Gothenburg, Medicinaregatan 1F, 413 90 Gothenburg, Sweden*

[2] *Laboratory of gene expression, Institute of Biotechnology AS CR, BIOCEV, Prumyslova 595, 252 50 Vestec by Prague, Czech Republic*

Myxoid liposarcoma (MLS) is characterised by the FUS-DDIT3, or the less common EWSR1-DDIT3 fusion oncogene and is the second most common type of liposarcoma. The fusion oncogenes encode chimeric transcription factors that are causal factors in tumourigenesis however, their functions are poorly known. Notwithstanding continuous progress in treating MLS patients, existing therapies suffer from a major flaw as they do not target the cancer stem cells (CSCs). Unique features of CSCs include self-renewal, tumour initiating capacity and increased resistance to radiotherapy- and chemotherapy-induced cell death. Thus, CSCs are crucial targets for successful therapy. MLS displays extensive intratumoural heterogeneity with distinct subpopulations of tumour cells but little is known about their features or roles in the tumour. The aims of this project were to define the role of fusion oncogenes in tumourigenesis and to define signalling pathways controlling CSC features in MLS. We investigated the regulatory mechanisms, expression levels and effects of FUS-DDIT3 in detail, and showed that FUS-DDIT3 was uniquely regulated at both transcriptional and post-translational level. Furthermore, we showed that MLS harbour subpopulations of cells with cancer stem cell properties and that their number is controlled by JAK-STAT signalling. Our single-cell data showed that individual cultured MLS cells expressed different amounts of canonical JAK-STAT transcripts. Our findings concerning FUS-DDIT3 function and CSCs have increased our molecular understanding of tumour development and therapy resistance in MLS that will facilitate development of specific treatment strategies.

P3: Establishing a new melanoma cell line from Circulating Tumor Cells (CTCs) from a patient with animal-type melanoma

Claudia Felici [1], Paola Cafforio [1], Francesco Mannavola [1], Stefania Stucci [1], Domenica Lovero [1], Francesco Silvestris [1]

[1] *Department of Biomedical Sciences and Human Oncology, University of Bari Aldo Moro, Bari Italy*

Introduction: Animal-type melanoma is a distinct melanoma subtype characterized by an indolent behavior. Since this is a rare phenotype of melanoma, there are not sufficient information regarding its biological characteristics. Aim The study describes the *in vitro* establishment of this variant of melanoma from CTCs and the subsequent phenotypic and molecular analysis.

Materials and methods: CTC isolation was performed by negative immunomagnetic separation using anti-CD45 microbeads. CTCs were then stabilized in culture by enriched medium replaced approximately every 3 days. Gene mutations on CTCs were analyzed using IonAmpliSeqCHPv2 and compared to the primary tumor tissue. Phenotypic characterization was assessed by flow cytometry and qPCR, whereas the clonogenicity of CTCs was confirmed by sphere-formation assay.

Results: Three patients were enrolled in the study but a single cell line was stabilized. After 30 day-culture, we observed a significantly high proliferation rate. We found a similar mutational status in CTC-derived cell line compared to the primary tumor, e.g. KRAS wild type and BRAF (V600E). The flow cytometry showed an intermediate epithelial/mesenchymal phenotype with high expression of E-cadherin, N-cadherin, CD105 and CD90. Furthermore, the CTC-linearized cells expressed stem cell markers such as ALDH1, CD44 and CD146, whereas both CD45 and CD34 as hematopoietic markers, were negative. qPCR data confirmed the cytofluorimetric analysis revealing the high expression of both epithelial (E-Cadherin) and mesenchymal (N-Cadherin, Snail, Twist) as well as stem cell (ALDH1, OCT3/4, NANOG) genes. Finally, we tested the self-renewal capability of these ALDH1+ cells with 3D assay and found that the proliferating cells were able to generate melanospheres.

Conclusions: Our data suggest that this CTC-derived cell line may be considered a starting point for an in-depth study on the biology of this atypical melanoma subtype. In addition, the expression of stemness markers hypothesizes that CTCs may represent a reservoir of cancer stem cells.

P4: Genome-wide study on Pol II stalling at promoter regions and its possible contribution to transcriptional consistency

Ana Hernández de Sande [1], Maria Bouvy-Liivrand [1], Tapio Vuorenmaa [1], Gerardo Del Muro Gónzalez [1], Aino Peltonen [1], Minna U. Kaikkonen-Määttä [2], Merja Heinäniemi [1]

[1] *School of Medicine, University of Eastern Finland, Kuopio, North-Savo 70200, Finland*

[2] *A. I. Virtanen Institute, University of Eastern Finland, Kuopio, North-Savo, 70200, Finland*

Introduction: Pol II stalling mechanism is defined by the accumulation of Pol II binding at promoter regions and can be monitored from cells by measuring active transcription. Genome-wide studies can reveal cell-specific regulatory programs that contribute to defining transcriptional states. Those states might differ among cellular populations, thus raising the need to study individual cell transcriptomes. Aim To elucidate the role of Pol II loading states in transcriptional variability across single-cell transcriptomes in context of stress responses.

Materials and Methods: We integrate nascent transcriptome data (Global Run-on sequencing, GRO-seq) to capture genome-wide Pol II distribution: Pol II loading at transcription start sites (TSS) and transcript elongation. To dissect the contribution of Pol II loading in transcriptional consistency, we collected single-cell data from endothelial cells subjected to heat shock stimuli. To account for technical variability, we have developed a protocol where we pool cells from different donors under different treatments in the same microfluidics plate. We then identify the origin of the cells computationally, using donor-specific SNP indexes and thus aim to account for the batch effect originated from single-cell isolation techniques.

Results: Using nascent transcriptomes (GRO-seq) we have defined genome-wide Pol II states in HUVEC cells at TSS. We have detected that those states are altered after subjecting the cell to stress stimuli such as heat shock, a well-known mechanism to release Pol II stalling, suggesting that they are subjected to regulation. Our preliminary analysis revealed an interesting juxtaposition: genes lacking Pol II pre-loading had significant enrichment of specific microRNA targets, indicating that two alternative approaches are utilized by cells to reduce transcriptional variability (noise-buffering).

Conclusion: Identification of Pol II states gives the first clues on how cells may regulate transcriptional consistency. However, there is a gap in our understanding regarding the role of such noise-filtering mechanisms during stress responses.

P5: Flexible Resolution CNV Profiling in Single Cells

Sarah M. Hücker [1], Jens Kunze [1], Christoph Klein [1,2], Stefan Kirsch [1]

[1] *Division "Personalized Tumor Therapy", Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Regensburg, Germany*

[2] *Experimental Medicine and Therapy Research, University of Regensburg, Germany*

Genome instability represents a hallmark of cancer. Therefore, the presence of copy number variations (CNVs) can be used to distinguish aberrant from normal cells. Usually, CNVs are characterized with the microarray based technique arrayCGH, however, besides high costs this method has the disadvantages that detectable CNVs are dependent on the oligonucleotides spotted and only a low number of samples can be processed in parallel. Also, whole genome sequencing can detect CNVs, but due to the fact that the costs of WGS are still relatively high, applying this method for CNV profiling is currently not reasonable. We have developed a cost-effective NGS method based on SMASH (Short Multiply Aggregated Sequence Homologies) to determine high-resolution CNV profiles of single cells: first, the whole genome of isolated single cells is amplified using the Ampli1TM WGA protocol following double strand synthesis with the Ampli1TM ds kit. Then, the resulting DNA is enzymatically fragmented into 50 bp pieces. Next, the small DNA fragments are stochastically concatenated, Illumina adapters are added, and a size selection is performed. The size selection is optimized for both MiSeq (2×300 bp) and HiSeq sequencing (2×150 bp). Thus, one read contains 6-12 loci, which highly reduces sequencing costs. For mapping of the chimeric reads and CNV detection, an in-house pipeline was used. To assess the performance of the method, the breast cancer cell line SKBR3 and as controls the lymphoblast cell line GM12878 and PBLs were used. As expected, the controls show balanced CNV profiles, whereas SKBR3 shows abundant DNA gains and losses in agreement to previously published aberrations. The resolution is dependent on sequencing depth and can go well below 100 kb. In summary, this new method is not only applicable for the identification of aberrant cells, but has the potential to characterize tumor heterogeneity with high accuracy.

P6: Resolving haematopoietic pathways in sea lamprey

Martin Kovář [1]

[1] *Department of cell differentiation, Institute of molecular genetics AVCR*

Haematopoiesis is one of the key features of vertebrates and its gene regulatory system is highly conserved thru the vertebrate phylum. But in the oldest living taxons of vertebrates it remains mostly unexplored. Haematopoiesis in higher vertebrates follow same rules and differentiation pathways which are controlled by conserved genes. We ask question if this is also case in the oldest living vertebrates. Therefore, we study sea lamprey to compare its haematopoietic process with the one of higher vertebrates. But long live and complex live cycle of those animals really limits gene manipulation technique and we must rely on explorative techniques. Fortunately new single cells sequenation techniques can help us to resolve complicated differentiation pathways in lamprey haematopoiesis.

P7: Spatial analysis of hypersensitive response to Potato virus Y in potato

Maja Križnik [1,2], Špela Baebler [1], Kristina Gruden [1]

[1] *Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia*
[2] *Jožef Stefan International Postgraduate School, Ljubljana, Slovenia*

Potato is one of the most important crop and Potato virus Y (PVY) is one of its most detrimental pathogens, causing crop losses worldwide. Potato cv. Rywal reacts to the virus by hypersensitive response (HR), where spot necroses that limit the virus at the site of virus entry are formed. NahG-Rywal plants, compromised in salicylic acid accumulation, develop necrotic lesions that do not limit viral spread and show delayed defence response. The mechanisms blocking viral multiplication and/or spread in HR are occurring close to the point of viral entry and are tightly spatially regulated. To investigate changes between potato cv. Rywal and transformed potato genotype NahG-Rywal, at molecular level, small tissue sections (consisting of few 10s of cells) at the site of virus multiplication and surrounding tissue were sampled and analyzed further by using improved RNA extraction methodology and high-throughput sequencing. We characterized and compared the gene and small RNA (sRNA) expression patterns between PVY-infected and healthy potato plants. A Rywal and NahG-Rywal sRNA-mRNA regulatory networks were constructed connecting sRNAs and their identified mRNA targets predicted *in silico* and using degradome-Seq approach. Integration of the sRNA expression and expression profiles of their targets confirmed many known, but also revealed some novel regulatory circuits associated mainly with immunity regulation and hormone signaling. Bioinformatic analysis identified several novel potato miRNAs. The search for PHAS loci identified several phasiRNAs, out of which some were also targeted immunity and hormone signaling genes and thus reinforcing the silencing effect on target mRNAs. Together, our findings revealed that both sRNA and mRNA expression patterns can be changed in response to PVY infection and they may regulate crucial responses in plant resistance to PVY. Our final challenge would be to integrate all the data and feed it to the potato defence model that we are developing.

P8: Single cell analysis of the endometrium

Julia Lipecki [1], Emma Lucas [1], Maria José Minhoto Diniz Da costa [1], Pavle Vrljicak [1], Jan Brosens [1], Sascha Ott [1]

[1] *University of Warwick*

The endometrium is a very dynamic tissue which undergoes great changes throughout the menstrual cycle. When progesterone levels increase following ovulation, the endometrium prepares the womb for pregnancy by transforming the endometrial stromal cells (EnSCs) into epithelioid decidual cells. These cells will play an important role in a successful pregnancy and will form the maternal part of the placenta. In the absence of pregnancy, diminishing progesterone levels will cause partial destruction and shedding of the tissue. In the clinic, recurrent pregnancy loss (RPL) patients are characterised by having three or more consecutive miscarriages, while *in-vitro* fertilisation (IVF) patients are characterised as those unable to conceive. We are interested in studying the endometrial tissue with the help of scRNA-sequencing technologies in both types of patients. We would like to identify cell types and corresponding marker genes for each patient group and compare the results between the two groups. Are we able to identify any similarities and/or differences in the composition of the endometrium tissue? What can we learn from scRNA-seq data about these two massively different conditions? In addition, we are also interested in characterising the temporal changes in the endometrium. We will work with in-vitro samples from a well-established decidualization technique and *in-vivo* endometrium biopsy samples. Which genes undergo temporal gene expression changes during the process of decidualization? Can we use these genes to accurately model the *in-vitro* decidualization time-points? How do the in-vivo samples map to the in-vitro model? Can we predict which day of the cycle the clinical samples come from? If so, how accurately can we do this and with what precision?

P9: Regulation of Noradrenaline Sensitivity of Human Mesenchymal Stromal/Stem Cells Associated with their Adipogenic Differentiation

Pyotr A. Tyurin-Kuzmin [1], Natalia I. Kalinina [1], Julia I. Fadeeva [1], Veronika Yu. Sysoeva [1], Vsevolod A. Tkachuk [1]

[1] *Department of Biochemistry and Molecular Medicine, Faculty of Medicine, M.V. Lomonosov Moscow State University, Moscow, Russia*

Introduction: Mesenchymal stromal/stem cells (MSC) are identified in the stromal-vascular compartment within the most of adult tissues including bone marrow, adipose tissue and skeletal muscles. MSC mediate physiological renewal of connective tissues by differentiation into multiple directions such as fat, bone and cartilage. MSC functions are under tight hormonal control, and noradrenaline is one of their most important regulators. Aim To define how sensitivity to noradrenaline of adipose derived MSC is regulated and is linked to differentiation properties of these cells.

Materials and Methods: Single cell analysis of calcium signaling, single cell analysis of adipogenic differentiation.

Results: We examined the mechanisms of regulation of MSC sensitivity to noradrenaline. Using flow cytometry and calcium imaging in single cells, we demonstrated that more than 80% of MSC expressed alpha1-adrenoceptors at their surface. However only 6.9±0.8% of MSC responded to noradrenaline by intracellular calcium release, therefore in the most of the cells alpha1-adrenoceptors were not coupled with Ca2+-dependent signaling. We showed that noradrenaline itself regulated MSC sensitivity to catecholamines by inducing the down-regulation of beta-adrenoceptors and heterologic sensitization of alpha1A-adrenoceptors. Noradrenaline-dependent intracellular signaling pathways were switched from cAMP-mediated to Ca2+-mediated ones. To evaluate how MSC responsiveness to noradrenaline is associated with differentiation properties of these cells we used live single cell imaging. First, we detected MSC responding to noradrenaline by calcium release. Then we induced adipogenic differentiation of these cel ls and we used live single cell imaging to track the fate of noradrenaline responding cells. We showed that although the most of MSC were differentiated into adipocytes, the cells responding to noradrenaline by calcium release never did.

Conclusions: Noradrenaline itself regulated responsiveness of individual cells to that hormone. Calcium response to noradrenaline was linked to adipogenic potential of individual cells.

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Poster session

New approaches, instruments for single cell analysis

P10: CTCelect-Instrument: Fully-Automated Enrichment, Detection and Isolation of CTCs

Sabine Alebrand [1], Christian Freese [1], Carmen Schwind [1], Jörn Wittek [1], Peter Spang [1], Knut Welzel [1], Michael Bassler [1]

[1] *Fraunhofer IMM, Carl-Zeiss-Str. 18-20, 55129 Mainz*

Single cell analysis in the context of liquid biopsy may offer a great opportunity for cancer diagnostics and therapy. In particular, the analysis of circulating tumor cells (CTCs) is of interest to predict the metastatic potential of tumors, cancer progression, and the chances of survival. In this context, we developed the CTCelect-instrument for the fully automated enrichment, detection and isolation of circulating tumor cells (CTCs) from a whole blood sample. The instrument consists of two modules, one for the enrich ment and staining of the CTCs and one for their detection and isolation. The modular design offers high flexibility in terms of potential applications. The CTCs are collected from whole blood samples by immunomagnetic enrichment and are afterwards specifically fluorescence labelled, which is required for their subsequent detection within a microfluidic chip. Finally, each detected CTCs is dispensed to a well of a microtiter plate and is available for subsequent analysis. The performance of the CTCelect-instrument was characterized by determining the cell recovery rate using MCF7 cells. The recovery rates for the immunomagnetic enrichment, the cell detection and the cell dispensing were determined as 93%, 90% and 86%, respectively. The probability to dispense a leukocyte in tandem with a CTC was estimated to be less than 10%. Furthermore, it was exemplarily demonstrated that the dispensed living cells can still be used for single cell PCR investigations. In summary, the CTCelect-instrument shows very promising results in terms of CTC detection and isolation for further single cell analysis. By changing the biological assay for the enrichment and staining, the CTCelect-process can be adapted to various cancer types/rare cells, making the CTCelect-instrument a very useful tool for single cell research applications.

P11: Development of High Spatial Resolution Stigmatic Imaging Mass Spectrometer

Jun Aoki [1], Hisanao Hazama [1], Toyoda Michisato [1]

[1] *Osaka University*

Measurement methods of spatial distribution of molecules at cellular-scale are required in many fields. Recently, scanning type imaging mass spectrometry with matrix-assisted laser desorption/ionization is intensively used for biomolecular analysis. However, the spatial resolution of scanning MALDI-IMS is limited to about 10 - 100 µm and inadequate for cellular-scale observation. Therefore, we are developing a stigmatic MALDI imaging mass spectrometer to achieve spatial resolution of sub-micron. The experimental apparatus for stigmatic imaging consists of a MALDI ion source with focusing ion optics, TOF mass spectrometer and a time and position sensitive ion detector. Sample molecules ionized by laser irradiation are extracted and accelerated by extraction electrode. Then ion distributions at the sample plate are magnified and projected with the ion optical lens system onto the detector. We observed grid pattern formed by test samples of crystal violet dye covered with fine metal mesh to evaluate imaging quality of our apparatus. This evaluation experiments verified that this apparatus achieved high spatial resolution of 1 micro-meter with a field of view of 500 x 500 micro-meter within observation time of several minutes. This spatial resolution and measurement speed was drastically improved from conventional scanning type imaging mass spectrometers. We also show applications of this apparatus to actual biological samples.

P12: Automation of Ampli1™ LowPass library preparation: streamlined strategy to unravel the heterogeneity at single cells level

Marianna Garonzi [1], Edoardo Petrini [1], Alberto Ferrarini [1], Paola Tononi [1], Valentina del Monaco [1], Rui P.L. Neves [2], Claudio Forcato [1], Genny Buson [1], Nikolas H. Stoecklein [2], Nicolò Manaresi [1]

[1] *Menarini Silicon Biosystems, Bologna, Italy*

[2] *Department of General, Visceral and Pediatric Surgery,University Hospital and Medical Faculty of the Heinrich-Heine University Düsseldorf, Düsseldorf, Germany*

Introduction: Single-cell low-pass Whole-Genome Sequencing (WGS) is an efficient method to study copy-number alterations (CNAs) and tumor heterogeneity. However, to properly deconvolve an heterogeneous sample, many individual cells need to be analysed.
Aims: We took advantage of STARlet Liquid Handling Robot (Hamilton®) to implement a streamlined, fully automated workflow that can process Ampli1™ WGA (Whole Genome Amplification) Kit (Menarini Silicon Biosystems) DNA products into sequencing-ready WGS libraries. We applied this method for the profiling of CNA in Circulating Tumor Cells (CTCs).
Materials and Methods: Single CTCs from 3 patients with metastatic castration-resistant prostate cancer (mCRPC) were enriched by CELLSEARCH® and isolated with the DEPArray™ system, then their genomes amplified using Ampli1™ WGA kit (Menarini Silicon Biosystems). Amplification products were processed with Ampli1™ LowPass kit (Menarini Silicon Biosystems) either manually or through the automated procedure. Resulting libraries were sequenced on MiSeq instrument (Illumina®), then CNA analysis was performed using Control-FREEC software.
Results: Hamilton-automated procedure generated 192 high quality WGS libraries with a hands-on time of 1.5 hours and a global turn-around time of two days. The same time was needed to manually generate only 30 libraries, of which 4 hours hands-on. Samples processed with both methods were highly similar in terms of sequencing metrics and showed about 90% of overlap in terms of CNAs detected. Clustering analysis showed that variability among samples is independent from the method used, indicating that Hamilton-processed samples were qualitatively indistinguishable from manually-generated ones.
Conclusion: Our data shows that automated Ampli1™ LowPass protocol generates WGS libraries qualitatively indistinguishable from our manual workflow. Ampli1™ LowPass automation on Hamilton STARlet it is a very cost-effective approach and can be run with high degree of robustness, repeatability and reproducibility. All these features are especially required to implement the Ampli1™ LowPass workflow in a clinical setting, for diagnostic applications.

P13: Advanced whole genome amplification technology enhances uniformity after storage and transport of single cells

Ruth Kläver [1], Andrea Janosch [1], Dorothee Unt [1], Samuel Rulli [2], Christian Korfhage [1], Norbert Hochstein [1]

[1]QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

[2]*QIAGEN Sciences Inc., 6951 Executive Way, Frederick, MD 21703*

The analysis of single cells is a fast growing field and many researchers are interested in various downstream analyses of an individual single cell. Whole genome amplification (WGA) overcomes the limitation of sample DNA from a single cell enabling multiple downstream applications. In many cases the WGA is not performed directly after collecting the single cell and often samples have to be shipped between the place of single cell isolation and the laboratory performing the WGA and downstream analysis. To maintain high uniformity of amplification (even after 14 days of storage at -20 °C between isolation and amplification) we developed an advanced version of the Phi-29 amplification system and added a single cell storage buffer optimized for the storage and transport of eukaryotic single cells.

To prove the positive effect of the advanced WGA and the new storage buffer on uniformity of amplification after storage of single cells, we isolated single Jurkat cells using a single cell capture devise (QIAscout technology) and released each isolated single cell either in 4 µl PBS or 4 µl of the advanced single cell storage buffer. Isolated single cells were processed directly after isolation or stored either for 2 days or 2 weeks at -20 °C. Afterwards success and uniformity of amplification were evaluated by qPCR using different single and multi copy genes and by targeted NGS analysis using a QIAseq targeted DNA Panel.

Results of qPCR clearly show that storage of single cells in the new single cell storage buffer in combination with the use of the advanced WGA leads to less variation in the amplification of different eukaryotic single cells. This high inter-sample uniformity was confirmed by subsequent targeted NGS. In addition, NGS analysis demonstrated a more uniform amplification of the single cell genome (intra-sample uniformity) when using the advanced WGA and optimized single cell storage buffer.

P14: A microfluidic device for investigation of cellular migration

Karel Klepárník [1], Vojtěch Ledvina [1]

[1] *Institute of Analytical Chemistry, Czech Academy of Sciences, Brno, Czech Republic*

Functionality of a microfluidic device made of polydimethylsiloxane for testing epithelial-mesenchymal transition of cancer cells is presented. Arrays of micropillars as obstacles with different spacing are a prerequisite of high throughput measurements and standard evaluations of cellular deformability and migratory activity. The miniaturization of the device with the total migration path length less than 1 mm significantly shortened analysis time. The device is compatible with fluorescence microscope in a time-lapse setup to visualize variability of mechanical properties of many individual cells under identical conditions. Cells were monitored for several hours.
The research was supported by Grant Agency of the Czech Republic, project No. 17-01995s.

P15: Circulating tumor cells (CTCs) in NSCLC incorporate differential subsets including the epithelial-mesenchymal transition (EMT) phenotype

Domenica Lovero [1], Francesco Mannavola [1], Paola Cafforio [1], Claudia Felici [1], Raffaele Palmirotta [1], Francesco Silvestris [1]

[1] *Department of Biomedical Sciences and Human Oncology, University of Bari Aldo Moro, Bari Italy*

Introduction: Non-small cell lung cancer (NSCLC) is a major metastatic tumor for its ability to spread out and generate metastases in distant organs. Recent evidence however suggests that the invasive phenotype of NSCLC is prevalently associated with the epithelial-to-mesenchymal transition (EMT) markers. Aim This study was devoted to improve a DEPArray cell separation protocol by using EMT markers to isolate CTCs for subsequent molecular analyses by Next Generation Sequencing (NGS).

Materials and methods: Blood samples from 21 NSCLC patients were depleted of CD45pos leukocytes and stained with an antibody panel to EMT markers. Cell sorting was performed by DEPArray equipment and the recruited CTCs were subjected to NGS analysis avoiding previous whole genome amplification (WGA) using the Ion AmpliSeq™ Cancer Hotspot Panel v2 on the Ion Torrent PGM™ system and compared to FFPE tumor tissue.

Results: Four CD45neg cell subsets were identified in all patients. Briefly: 1) cells expressing only epithelial markers (E-CTC); 2) cells co-expressing both epithelial and mesenchymal markers (EM-CTC); 3) cells expressing only mesenchymal markers (MES-CTC); 4) cells negative for both phenotype markers (NEG-CTC). MES-CTC was the most represented subset (50.6%±2.8%) thus supporting the role of EMT in the early phase of tumor spreading. Mutational analysis of MES-CTCs from 5 patients identified 19 sequence variants in 11 genes, showing either inter- or intra-patient heterogeneity (CTCs vs FFPE). Furthermore, at least one pathogenic variant was identified in all CTCs.

Conclusions: Our data support the suitability of the liquid biopsy in NSCLC patients and confirm the intra-tumor heterogeneity occurring in different patients. Moreover, the classification of CTCs by EMT markers may characterize different CTC subsets that would be lost when using other CTC separation methods including the EPCAM-based recognition by CellSearch technology.

P16: Accurate Single Cell mutation detection by Targeted next-generation sequencing profiling to resolve tumor heterogeneity

Edoardo Petrini [1], Marianna Garonzi [1], Alberto Ferrarini [1], Paola Tononi [1], Valentina del Monaco [1], Claudio Forcato [1], Genny Buson [1], Nicolò Manaresi [1]

[1] *Menarini Silicon Biosystems, Bologna, Italy*

Introduction: Hotspot mutations are important markers to evaluate tumor heterogeneity, however low input material obtained from single-cells is unsuitable for DNA library preparation, hence for mutations detection. Therefore, single-cell DNA is usually amplified through PCR, which can introduce biases and jeopardize subsequent analyses.
Aims: We exploited the deterministic nature of Ampli1™ WGA (Whole Genome Amplification) Kit (Menarini Silicon Biosystems), the best-in-class WGA method in terms of low allelic drop-outs and low amplification biases, to design a targeted cancer panel. The panel covers 60 clinically-relevant genes, including more than 1500 mutation hotspots and copy-number alterations (CNAs) for a subset of 19 genes.
Materials and Methods: Three single cells and 1 bulk sample from neuroblastoma, lung adenocarcinoma cell lines and white blood cells were isolated using DEPArray NxT™ platform, for a total of 12 samples. Isolated cells underwent the Ampli1™ WGA procedure, and further processed by Ampli1™ ds/ReAmp kit. Ten nanograms of WGA dsDNA product were used as starting material for the Ampli1™ targeted cancer panel and resulting DNA libraries were sequenced on Illumina® MiSeq platform (at least 1 million reads per samples). Sequences were aligned to hg19 genome, variant calling was performed using LoFreq package.
Results: Overall, bioinformatic analysis showed that on-target reads were above 95% for all samples, with 95% of targeted amplicons covered with a read depth of at least 100x. The Ampli1™ targeted cancer panel correctly identified clinically relevant mutations, such as EGFRdel E746A750 and ALK F1174L. Those mutations have been shown to be important therapeutic indicators and are associated with lung adenocarcinomas and neuroblastomas, respectively.
Conclusion: The Ampli1™ targeted cancer panel allows fast and accurate identification of clinically relevant mutations at single cell level, including SNPs, INDELs and CNAs.

P17: A New Way Of Filtering DropSeq Data

Annika Stechemesser [1], Rachel Pounds [2], Emma Lucas [3], Pavle Vrljicak [3], Julia Lipecki [3], Jan Brosens [3], Sean Kehoe [2], Christopher Dawson [2], Sascha Ott [3], Jason Yap [2], Lawrence Young [4], Jianmin Zuo [2], Paul Moss [5]

[1] *Centre for Complexity Science, University of Warwick*

[2] *Institute of Cancer & Genomic Sciences, University of Birmingham*

[3] *Department of Computer Sciences, University of Warwick*

[4] *University of Warwick Medical School*

[5] *Institute of Immunology and Immunotherapy, University of Birmingham*

In every analysis of DropSeq data, filtering is the first crucial step to enable a meaningful downstream analysis. Broken cells, doublets or empty reads can lead to obscured results, meaning cell types don't get identified correctly or get missed completely. Especially for the analysis of cancerous tissue this can have fatal consequences.
We discuss the state of the art of cell filtering and introduce a new, algorithmic method to filter out "noisy" cells. A comparison between the new method and standard methods is made by applying them to different datasets, revealing hidden cell types.

P18: From single cell to mutation status in metastases: heterogeneity of circulating tumor cells (CTCs) in advanced breast cancer patients.

Małgorzata Szostakowska [1], Ewa Grzybowska [1], Anna Fabisiewicz [1]

[1] *Department of Molecular and Translational Oncology, The Maria Sklodowska-Curie Cancer Center and Institute of Oncology, Warsaw, Poland*

Introduction: Current adjuvant treatment strategies are guided by characterization of the primary tumor, despite the f act that metastases can differ in terms of genetic characteristics. Thus, there is a need for molecular characterization of the metastatic lesion. However, the inaccessibility of the metastatic sites and invasiveness of biopsy is challenging. Therefore, analyzing circulating tumor cells (CTCs) as a surrogate for the metastatic lesion would be non-invasive method for determination of heterogeneity of tumor cells among primary tumor and metastases. Tumor progression is associated with resistance to treatment and cells capability to migration. Identification of resistance associated mutations in CTCs may improve treatment decisions and patient outcome.

Aim: Identification of mutational changes in single CTCs in metastatic breast cancer associated with resistance to anti-estrogen treatment and cell migration. Comparison of molecular profiles in primary tumor and metastases.

Materials and Methods: Primary tumors and peripheral blood (PB) samples were collected from patients with advanced breast malignancy during treatment with anti-estrogen therapy, in The Maria Skłodowska-Curie Institute of Oncology, in Warsaw. Nuclear cells were isolated and prepared for further analysis. Identification, enumeration and isolation of CTCs form PB samples was performed using novel instrument CytoTrack (Denmark). Single CTCs isolated via micromanipulation (CytoPicker) were lysed and single cell whole genome amplification (WGA) was performed using MALBAC system. Mutations in genes associated with anti-estrogen resistance and migration (ESR1, ESR2, AKT1, AKT2, PIK3CA) are going to be identified via NGS and compared with mutation status from primary tumor samples.

Results: Our preliminary data show utility of CytoTrack for identification, enumeration and isolation of single CTCs from breast cancer patients. The studies of CTCs heterogeneity are ongoing project.

Conclusion: CytoTrack enables to isolate CTCs independently of their EpCAM status.

Poster session

Single cell diagnostics

P19: Monitoring of AR-V7 expression in castration-resistant prostate cancer patients using circulating tumour cells

Katarina Baluchova [1], Boris Elias [2], Jan Kliment Jr. [2], Jan Luptak [2], Jan Kliment [2]

[1] *Division of Oncology, Biomedical Center Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia*

[2] *Department of Urology, Jessenius School of Medicine, Comenius University, Martin, Slovakia*

Introductions and Objectives. Prostate cancer (PC) is the second most frequently diagnosed oncological disorder in Slovakian men and third leading in numbers of associated deaths. Initial diagnosis relies on physical, histological, biochemical or radiological examinations and more recently on molecular profiling of tumour cells. Notably, androgen receptor splice variant 7 (AR-V7) serves as a biomarker predicting the sensitivity and resistance to abiraterone/enzalutamide and taxanes in patients with castration-resistant PC (CRPC). The former are used for targeted inhibition of AR signalling pathway that adapts during the course of androgen deprivation therapy (ADT) and taxanes for broad induction of mitotic arrest.

Materials and Methods. AR-V7 expression status was investigated in 71 men with CRPC using circulating tumour cells (CTC) isolated with the Adnatest® system from peripheral blood prior to treatment initiation. EpCAM- and/or HER2-positive CTC were recorded, if at least one of the genes PSA, PSMA and EGFR were transcribed as evidenced by RT-PCR on the Agilent 2100 Bioanalyser.

Results. Forty CTC-positive patients (56.4 %) with frequencies of PSA, PSMA and EGFR expression of 95.0 %, 57.5 % and 15.0 % respectively were further assessed for expression of AR and AR-V7 specific regions spanning exon 2, 3 and the cryptic exon 3. Twenty-seven patients had AR (67.5 %) and eleven had AR-V7 (27.5 %) expressed in CTC. Currently, these men are receiving docetaxel and are being monitored for AR-V7 status using CTC.

Conclusions. CRPC patients expressing AR-V7 in CTC of 27.5 % in Slovakian cohort of 71 were indicated for treatment with taxanes rather than the AR signalling inhibitors. Clinical data collected prior treatment initiation, at therapy response and at disease progression are being evaluated alongside the test detecting AR-V7 status in CTC.

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P20: Trop-2 heterogeneity and plasticity in cancer

Lucia Binó [1,2], Ján Remšík [1,2,3], Vojtěch Dvořák [1,3], Zuzana Kahounová [1,2], Gvantsa Kharaishvili [4], Šárka Šimečková [1,2,3], Radek Fedr [1,2], Tereza Kučírková [2,3], Sára Lenárt [3], Ximena Maria Muresan [1,2], Eva Slabáková [1], Lucia Knopfová [2,3], Jan Bouchal [4], Milan Král [5], Petr Beneš [2,3], Karel Souček [1,2]

[1] *Department of Cytokinetics, Institute of Biophysics of the CAS, v.v.i., Brno, Czech Republic*

[2] *Center of Biomolecular and Cellular Engineering, International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic*

[3] *Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic*

[4] *Department of Clinical and Molecular Pathology, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic*

[5] *Department of Urology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic*

Dissemination of cancer cells to the distant organs has fatal consequences to the patients with malignant tumors. During cancer metastasis, malignant cells display phenotypic plasticity associated with the epithelial-to-mesenchymal transition (EMT). Release of circulating tumor cells - CTCs into the bloodstream has a key role in this process. The cell-surface glycoprotein Trop-2 is commonly overexpressed in carcinomas and its deregulation is proposed to be associated with cancer progression and poor prognosis thus it represents an exceptional target for therapy. We aimed to inspect the plasticity of primary tumors and its reflection in the plasticity of the secondary metastasis and the populations of CTCs with a special interest in Trop-2. Using flow cytometry, qRT-PCR, microscopy and publicly-available databases we analyzed the EMT status and Trop-2 expression in breast and prostate cancer cell lines and patient samples. To examine alterations of Trop-2 expression during dissemination, we analyzed Trop-2 bulk and single-cell expression in primary tumors, CTC-enriched fractions, and lung macrometastases developed in orthotopic-injected mouse model. We demonstrate that surface Trop-2 associates with an epithelial phenotype and correlates with E-cadherin expression in cancer cells, *in vitro*, *in vivo* and in patient samples. We show Trop-2 heterogeneity is induced after orthotopic inoculation. Likewise, heterogeneity in Trop-2 expression in patient samples illustrates the biological significance of Trop-2 expression plasticity. Interestingly, in mouse model, Trop-2 mRNA was detected in all three tissue types without statistically-significant difference. Our results indicate Trop-2 is heterogeneously expressed throughout the metastatic cascade and indicates that Trop-2 downregulation is not necessary for the process of metastasis.

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P21: White blood cells genomic profiles in patients with advanced colorectal cancer

Pavel Ostašov [1,2], Jana-Aletta Thiele [1], Peter Kuhn [3], Pavel Pitule [1,2]

[1] *Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Czech Republic*

[2] *Department of Embryology and Histology, Faculty of Medicine in Pilsen, Charles University, Czech Republic*

[3] *The Bridge Institute, Dornsife College of Letters, Arts and Sciences, University of Southern California, USA*

Introduction: Colorectal cancer is at the forefront of incidence and mortality associated with cancer globally as well as in Czech Republic. Appropriate prognostic and predictive markers are required to select the optimal treatment schedule and liquid biopsy of free nucleic acids and circulating tumor cells is being made to use. However, in most cases the analysis is focused on finding tumor-like cells and little attention is given to white blood cells. Aims In this work we decided to explore genomic profile of individual white blood cells from patients with colorectal cancer to find out if it is possible to identify features related to patient outcome.

Materials and Methods: We analyzed a total of 52 white blood cells from 21 patients with colorectal carcinoma. These white blood cells were collected by the micromanipulator from preparations of the peripheral blood nuclear cell suspensions that were used to detect circulating tumor cells. For individual white blood cells, their DNA was amplified using the SigmaAldrich WGA4 kit, the New England Biolabs NEBNext Ultra library was prepared and sequenced on the HiSeq instrument. By analyzing copy number variations in combination with the C5.0 algorithm, we were able to identify a region whose aberrations in white blood cells appear to be associated with duration of tumor-free survival.

Results: We found out that most of the potential signals are caused by sample processing. However, we were able to identify a region in the genome of white blood cells whose aberrations are associated with duration of tumor-free survival. No association was found with overall survival.

Conclusions: It seems that genome of white blood cells of colorectal patients contains information that might be associated with patients disease free survival. The biological relevance and mechanism are yet to be studied.

Study was supported by projects Progres Q39, UNCE/MED/006 and LO1503

P22: Genomic profiling of circulating tumor cells in stage IV colorectal cancer

Thiele J.A. [1], Sandstrom Gerdtsson [2], Rappard K. [2], Ostasov P. [1], Liska V. [1,3], Vycital O. [1,3], Bruha J. [1,3], Hosek P. [1], Kralickova M. [4], Kuhn P. [2], Hicks J. [2], Pitule P. [1]

[1] *Biomedical Center, Faculty of Medicine in Pilsen, Charles University*

[2] *The Bridge Institute, Dornsife College of Letters, Arts and Sciences, University of Southern California*

[3] *Department of Surgery, Faculty of Medicine in Pilsen, Charles University*

[4] *Department of Embryology and Histology, Faculty of Medicine in Pilsen*

Introduction: Circulating tumor cells (CTCs) represent a crucial step in the metastatic cascade. In several cancer types, like breast or prostate, their enumeration might be sufficient for disease prognosis. However, to use more of their diagnostic potential, it is advantageous to employ molecular and genomic profiling for further analysis of detected CTCs. Aims Goal of the study was to characterize CTCs in patients with advanced CRC and to describe clonal relationships between CTCs, primary tumor tissue and liver metastases.

Materials and Methods: We analyzed a total of 47 patients with stage IV CRC. CTCs were detected by HD-SCA platform. Selected CTCs were extracted from the microscopy slide, their DNA was amplified by SigmaAldrich WGA4 kit, sequencing library was prepared by New England Biolabs NEBNext Ultra kit and sequenced on the HiSeq instrument. Copy number variations profiles were used for identification of clonality among cells.

Results: Our data showed no clonality within the population of CTCs from individual patients, neither identified similar changes among the patient cohort. We were able to detect similar clones within the tissue samples of primary tumor and liver metastasis.

Conclusions: Colorectal cancer CTCs are highly heterogeneous with very low or nonexistent clonality, which may complicate their further clinical applicability. Another future approach would be the analysis of genomic instability in CTCs instead of comparison of clonal profiles.

Support: This work has been supported by the Charles University Research Fund (Progres Q39), by Charles University Research Centre program UNCE/MED/006 "University Center of Clinical and Experimental Liver Surgery" and by the National Sustainability Program I (NPU I) Nr. LO1503 provided by the Ministry of Education Youth and Sports of the Czech Republic.

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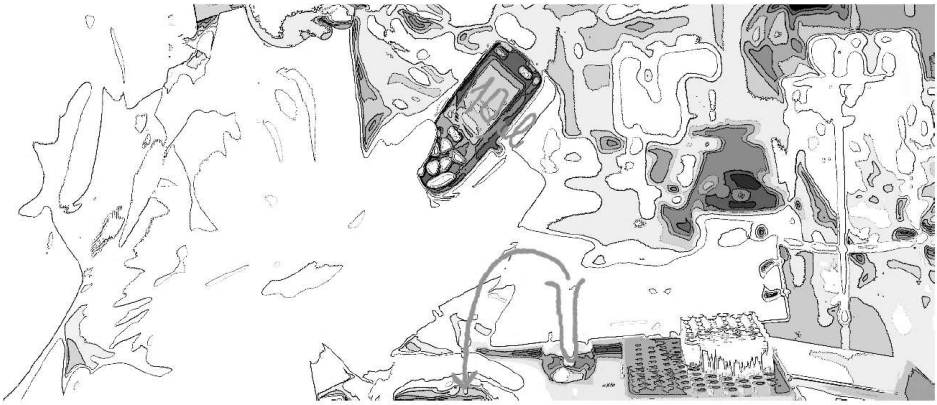

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Poster session

Other

P23: Multiplex plasma protein profiling in patients with familial hypercholesterolemia under different therapy

Dana Dlouha [1], Eva Rohlova [1,2,3,4], Milan Blaha [5], Jaroslav A. Hubacek [1], Vladimir Blaha [6]

[1] *Centre for Experimental Medicine - Laboratory for Atherosclerosis Research; Institute for Clinical and Experimental Medicine, Videnska 1958/9, Prague 140 21, Czech Republic*

[2] *Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague, Czech Republic*

[3] *Laboratory of Gene Expression, Institute of Biotechnology CAS, BIOCEV, Vestec, Czech Republic*

[4] *TATAA Biocenter AB, Gothenburg, Sweden*

[5] *4th Department of Internal Medicine, Charles University School of Medicine and Teaching Hospital, Hradec Králové, Czech Republic*

[6] *Department of Gerontology and Metabolism, Charles University School of Medicine and Teaching Hospital, Hradec Králové, Czech Republic*

Background: Familial hypercholesterolaemia (FH) is an autosomal-dominant disorder caused by mutations within the LDLR, APOB, and PCSK9 genes, characterized by high plasma levels of total cholesterol and low-density lipoprotein cholesterol (LDLc). LDL apheresis is a type of aggressive therapy, uses an extracorporeal circulation device to eliminate LDLc from plasma. The aim of the study was to compare concentrations of plasma CVD-related protein biomarkers in FH patients only under hypolipidemic treatment (before and 1 month under statin therapy), and under combined LDL apheresis/hypolipidemic treatment (before and after plasma lipoproteins removing).

Materials and Methods: We measured a profile of 184 CVD-related proteins using a novel sensitive and specific Proximity Extension Assay (PEA). Plasma samples were collected from FH patients under statins therapy only (N=23; men=11, age 57.2±12.3 years) and from patients under combined long-term LDL apheresis/statins therapy (N=16; men=8; age 57.0±13.6 years). The statistics were calculated using GenEx SW.

Results: Statins therapy only influenced in sum 43 plasma proteins involved mainly in inflammation, atherosclerosis, coronary artery disease, myocardial ischemia and muscle injury, regulation of coagulation and fibrinolysis. We found significantly increased levels e.g. of Interleukin-1 receptor family member (P<0.004), N-terminal of the prohormone brain natriuretic peptide (P<0.02), Myoglobin (P<0.03). Combined apheresis/hypolipidemic treatment significantly affected 44 plasma proteins mostly involved in the regulation of cholesterol homeostasis, the progression of atherosclerosis lesion, ischemia, and inflammation. We have observed an increase of the plasma concentrations of Osteopontin (P<0.0001) and a decrease of Low-density lipoprotein receptor (P<0.0001), Proprotein convertase subtilisin/kexin type 9 (P<0.0001) and Matrix metalloproteinase-3 (P<0.0002).

Conclusions: Using PEA protein profiling we detected that statins treatment influenced mainly plasma biomarkers participated in muscle injury, regulation of coagulation and fibrinolysis. Combined LDL apheresis/hypolipidemic therapy had an effect on plasma levels of many proteins involved in the regulation of cholesterol metabolism, angiogenesis, myocardial ischemia, and atherosclerosis progression.

P24: BIOCEV GeneCore – More than just gene expression profiling

Filip Franko [1], Lucie Langerová [1], David Švec [1,2]

[1] *Gene Core BIOCEV, Průmyslová 595, Vestec 252 50, Czech Republic*

[2] *Laboratory of Gene Expression, Institute of Biotechnology CAS, BIOCEV, Průmyslová 595, Vestec 252 50, Czech Republic*

We present services of BIOCEV GeneCore - the best equipped core facility and service provider in the field of gene expression in Central Europe. We have broad experience in quality control (QC e.g. Fragment Analyser) in a single cell analysis (automated cell picking ALS Cellcelector), high-throughput and digital PCR (Fluidigm Biomark, BioRad QX200 Droplet Digital PCR System) and NGS library preparation.
We emphasise quality control, which is often neglected. Effective QC is based on the use of molecular tools to control contamination (RNA/DNA spikes), genomic background (ValidPrime) and quality of RNA (ΔAmp, RIN). We also take part in development of these methods to facilitate analysis of gene expression starting from bulk samples, down to the level of individual cells (direct lysis).
In addition to conventional qPCR analysis, we focus on single cells expression profiling and multi-analyte approach. Analysis of DNA/RNA/protein in parallel in one sample even on the single cell level provides comprehensive tool to map gene expression and characterizes types of cells and to determine the degree of differentiation and to study the pathological condition.
Currently, we offer assistance with library preparations and experimental design of RNA-Seq experiments, which are key preconditions for a successful project. And also new *Two-Tailed PCR* for ultrasensitive *analysis of microRNAs*. Based on an innovative novel design with a RT primer sensing the microRNA using two connected hemi-probes exceeding sensitivity and superior specificity is achieved.

P25: Evaluation of cytocompatibility of novel nHAp based on hydrogels functionalized with resveratrol using microRNA expression profiling and cytometry-based single cell approach

Agnieszka Smieszek [1,2], Justyna Trynda [1,2], Katarzyna Kornicka [1], Paulina Sobierajska [3], Sara Targonska [3], Rafal J. Wiglusz [3,4], Krzysztof Marycz [1,2]

[1] *Department of Experimental Biology, Faculty of Biology and Animal Science, Wroclaw University of Environmental and Life Sciences, ul. Norwida 27B, 50-375 Wroclaw, Poland*

[2] *Wroclaw Research Centre EIT+, Wrocław, Poland*

[3] *Institute of Low Temperature and Structure Research, Polish Academy of Sciences, Okolna 2, 50-422 Wroclaw, Poland*

[4] *Centre for Advanced Materials and Smart Structures, Polish Academy of Sciences, Okolna 2, 50-950 Wroclaw, Poland*

Evaluation of biomaterials cytocompatibility is usually based on determination of cells proliferate activity and morphology in cultures with tested scaffolds. However, in order to obtain more comprehensive information about cellular response to various biomaterials such studies should also include the analysis of the materials effect on cells at the molecular level. The aim of this study was to perform the complex characterization of biological properties and cytocompatibility of new multi-functional hydrogels prepared using 3,6-anhydro-α-L-galacto-β-D-galactan (Galactose hydrogel) and functionalized with hydroxyapatite nanoparticles as well as with three different concentrations of resveratrol (0.1, 0.5 and 1 uM). The research was designed to determine basic cytophysiological parameters of cells in cultures with novel scaffolds. The cytocompatibility of biomaterials was evaluated using model of human multipotent stromal cells derived from adipose-tissue (hASCs). The morphology of cells cultured on biomaterials was observed using scanning electron microscope and epiflurescence microscope. The metabolic activity of hASCs cultures was determined based on Alamar Blue assay. Moreover, population doubling time along with the cell adhesion rate was calculated. The cell cycle was determined based on PI staining, while apoptosis profile was evaluated with Annexin V-FITC/7-AAD-based assay. The expression of genes associated with cell proliferation and apoptosis was tested based on mRNA and miRNA level. Results: The profound screening test was used to select highly bioactive biomaterial, with the optimal concentration of resveratrol. The study of morphology, growth pattern and metabolic activity of cells indicated that all tested biomaterials were cytocompatible. However, the cytometry-based analysis of cell cycle distribution as well as apoptosis, allowed to perform more precise assessment of cytotoxic effect of tested scaffolds. The results of the analysis enabled indicating scaffold doped with 0.1 uM resveratrol as the most cytocompatible biomaterial. The results were reflected with the miRNA expression profiles, especially with the increased level of miR-145 and mir-320, which are important for multipotent stromal cells proliferation and differentiation. The analysis of miRNA expression, cell distribution in cell cycle and apoptosis profile may contribute to more in-depth evaluation of biomaterials cytocompatibility.

P26: Metformin as an agent modulating proliferative activity of adipose-derived multipotent stromal cells isolated from horses with equine metabolic syndrome.

Agnieszka Śmieszek [1,2], Justyna Trynda [1,2], Jolanta Szłapka-Kosarzewska [1], Katarzyna Kornicka [1], Peter Androvič [3], Lukas Valihrach [3], Lucie Langerová [4], Krzysztof Marycz [1,2]

[1] *Department of Experimental Biology, The Faculty of Biology and Animal Science, Wroclaw University of Environmental and Life Sciences, Norwida 25, 50-375 Wroclaw, Poland*

[2] *Wroclaw Research Centre EIT+, Stablowicka 147, 54-066 Wroclaw, Poland*

[3] *Laboratory of Gene Expression, Institute of Biotechnology CAS, Biocev, Průmyslová 595, Vestec 252 50, Czech Republic*

[4] *Gene Core BIOCEV, Průmyslová 595, Vestec 252 50, Czech Republic*

Metformin is predominantly used for treatment of human type 2 diabetes or prediabetes conditions. MET belongs to the group of insulin-sensitising drugs and therefore is considered as an agent for the treatment of equine metabolic syndrome (EMS). It was also shown that MET may affect the proliferative activity of multipotent mesenchymal stromal cells (MSCs), widely applied both as autologous and allogenic transplants, in human and veterinary regenerative medicine, e.g. for the treatment of orthopaedic conditions. After concluding our previous studies showing that multipotent stromal cells isolated from adipose tissue of horses affected by EMS (EqASCEMS) are characterized by decreased proliferative activity and viability, we started to seek for bioactive agents that would positively influence their cytophysiology in vitro. Thus, the aim of the study was to investigate the role of metformin on proliferative status of EqASCEMS. **Materials and methods:** The EqASC isolated from healthy (EqASCHE) and EMS horses were treated with 0.5 uM of metformin for 72 hours. The modulatory effect of metformin on proliferation of EqASC was investigated using BrdU assay, while the metabolic activity was tested using Alamar Blue assay. Additionally, the influence of metformin on cell cycle state was analysed. Mitochondrial membrane potential as well as viability of cells were monitored using flow cytometry based approach (Muse® Cell Analyzer, Merck). The mRNA level of genes involved in the apoptosis was determined using RT-qPCR technique, while the profile of chosen miRNA molecules was investigated using Two-tailed RT-qPCR method. The accumulation of Wnt3a and b-catenin was measured by Western blotting. The population doubling time in EqASCs cultures treated with MET decreased. Metformin improved mitochondrial potential and viability of investigated populations of EqASCs. The Bax/Bcl-2 ratio determined on mRNA level was decreased, which indicates anti-apoptotic character of metformin toward EqASCs. Additionally, cultures treated with metformin expressed increased level of Wnt-3a and b-catenin. Moreover, miR-16-5p, miR-21-5p, miR-29a-3p, miR-140-3p and miR-145-5p were noted to be up-regulated after metformin treatment. Metformin proved to be improving the viability, proliferative and metabolic status of EqASC, derived both from healthy, as well as EMS horses.

P27: Prognostic and predictive markers of breast cancer on the basis of molecular profiling

Viktor Hlavac [1,2], Radka Vaclavikova [1,2], Marie Ehrlichova [1,2], Karolina Seborova [1,2], Veronika Brynychová [1,2], Vaclav Pecha [3], Jiri Gatek [4], Pavel Soucek [1,2]

[1] *Department of Toxicogenomics, National Institute of Public Health, Prague, Czech Republic*;

[2] *Laboratory of Pharmacogenomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic*

[3] *Department of Oncosurgery Medicon, Prague, Czech Republic*

[4] *Department of Surgery, Hospital Atlas, Zlín, Czech Republic*;

Introduction: Breast cancer is the most common cancer in women worldwide. One of the obstacles to successful treatment is a multidrug resistance. Cytochromes P-450 (CYPs) contribute to the resistance by metabolizing of anticancer drugs in liver and tumor cells. Membrane ATP-binding cassette (ABC) and solute carrier (SLC) transporters may confer to drug sensitivity by transporting drugs across cell membrane. The aim of our study: comparison of gene expression levels of such genes in tumor and adjacent tissues of breast cancer patients with their clinical prognostic features and to study the genetic variability in blood samples of the patients.

Materials and Methods: Gene expression profile (n = 118 for CYPs and n = 83 for transporters) was determined using real-time PCR on ViiA7 machine. Genetic variability of candidate genes was estimated using the next-generation sequencing (NGS) by the help of MiSeq System (n = 105).

Results: The majority of genes were deregulated in tumors compared with non-tumor control tissues. We found significant associations of particular CYPs and membrane transporter genes (e.g. CYP2B6, CYP3A4, CYP2W1, SLC46A1, SLCO1A2, SLC19A1, ABCAs, ABCC8, ABCD1/2) with breast cancer prognosis and response to chemotherapy. We developed a panel of genes for NGS using target enrichment of exons of the candidate genes and successfully sequenced so far 105 patients. Genetic variants found were prioritized by in-silico methods and compared with clinical features of the patients.

Conclusion: NGS is plausible method for the assessment of variability and the effect of candidate genes, which were found to be associated with breast cancer prognosis and therapy outcome. Supported by the research grants P303/12/G163 of the Czech Science Foundation, National Center of Medical Genetics, project no. CZ.02.1.01/0.0/0.0/16_013/0001634 and AZV project 15-25618A.

P28: Single-cell studies: Focus on reverse transcription

Zucha Daniel[1,2], Androvic Peter[1,3], Kubista Mikael[1,4], Valihrach Lukas[1]

[1] *Laboratory of Gene Expression, Institute of Biotechnology CAS, BIOCEV, Vestec, Czech Republic*

[2] *Faculty of Science, Charles University, Prague, Czech Republic*

[3] *Faculty of Science, Palacký University, Olomouc, Czech Republic*

[4] *TATAA Biocenter, Gothenburg, Sweden*

In the field of gene expression research, the reverse transcription (RT) is often the initial enzymatic reaction when processing the samples. However, it is known that the reaction output is far from ideal, as it relies on the choice of priming strategy, target template and reverse transcriptase used.

The aim of this study was to compare the sensitivity, accuracy, reproducibility and yield of 11 commercially available reverse transcriptases with varying origin, RNase H activity and price. This was tested under 2 priming strategies – mixture of random hexamers with oligo(dT)$_{15}$ and oligo(dT)$_{15}$ - targeting transcripts with abundancy ranging from 22 to 282,000 copies per RT reaction.

Even though all reverse transcriptases performed with great accuracy and reproducibility, enzymes highly varied in their ability to transcribe low abundant template. Similarly, differences were observed between enzyme yields, where two enzymes – SuperScript IV and Maxima H- - showed significantly better results amongst all the enzymes tested when the reaction was primed by mixture of random hexamers and oligo(dT)$_{15}$. Additionally, it has been observed that position of qPCR primers plays a role in the outcome of the experiment. All 11 reverse transcriptases reported consistently decreasing reaction yield with increasing distance from RNA sequence 3'end. This effect is more significant for oligo(dT)$_{15}$ priming strategy. For mixture of random hexamers and oligo(dT)$_{15}$ is less pronounced, however still observable.

Our results showed that some reverse transcriptases are more suitable for single-cell reverse transcription than others. Additionally, our findings suggest that for the purpose of reliable transcript quantification, it is advantageous to design qPCR primers to the proximity of 3'end of RNA sequence. This work may thus serve as a guideline for the improvement and development of new single-cell quantification protocols.

P29: Single cell ICP-MS: Quantification of metal content in individual cells - An insight into cancer treatment

Helmut Ernstberger[1], **Chady Stephan**[1], **Ruth Merrifield**[1]

[1] *PerkinElmer*

Cancer therapy drugs are usually administered at high doses to have the desired effect on cancer cells; however this can lead to long and short term side effects, and cellular resistance to the drug. Thus, there is a need for targeted cancer therapy drugs to be developed. Quantifying the uptake and effect of targeted drugs has been impossible, traditional methods for testing drug uptake into cells consist of total digestion of the cell population, losing vital information on the mass of drug per cell and the percentage/number of cells containing the drug. Here, we present a new te chnique, Single Cell ICP-MS (SC-ICP-MS), which is capable of quantifying this. We use the uptake of the chemotherapy drug, cisplatin, into the resistant and non-resistant (A2780 and A2780-CP70) ovarian cancer cell lines. SC-ICP-MS analysis allowed for the quantitation of cisplatin, and other metals, within individual cells yielding information on mass and mass distribution. This technique would be a useful tool in the development of targeted drugs giving fast and in-depth results on the amount of drug and drug distribution in cells that can be linked to biological cellular responses.

Participants list

Pavel Abaffy
Institute of Biotechnology CAS, v. v. i.
pavel.abaffy@ibt.cas.cz

Sabine Alebrand
Fraunhofer Institute for Microengineering and Microsystems
denise.schwab@imm.fraunhofer.de

Peter Androvic
Institute of Biotechnology CAS, v. v. i.
peter.androvic@ibt.cas.cz

Jun Aoki
Osaka University
ajunmail@mass.phys.sci.osaka-u.ac.jp

Katarina Baluchova
Biomedical Center Martin
baluchova@jfmed.uniba.sk

Pascal Barbry
Université Côte d'Azur
barbry@ipmc.cnrs.fr

Yassine Belloum
University Medical Center Hamburg-Eppendorf
y.belloum@uke.de

Šárka Benešová
Institute of Biotechnology CAS, v. v. i.
besarka16@gmail.com

Claudia Bevilacqua
INRA
claudia.bevilacqua@inra.fr

Lucia Binó
International Clinical Research Center FNUSA
lucia.bino@gmal.com

Vladimir Bobek
Faculty Hospital Kralovske Vinohrady
vladimir.bobek@fnkv.cz

Chiara Bolognesi
Menarini Silicon Biosystems
cbolognesi@siliconbiosystems.com

Vojtech Braborec
Institute of reproductive medicine and geneticss
v.braborec@ivf-kv.cz

Jillian Bracht
Pangaea Oncology
jill94bracht@gmail.com

Wolfgang Breitwieser
University of Manchester
wolfgang.breitwieser@cruk.manchester.ac.uk

Genny Buson
Menarini Silicon Biosystems
gbuson@siliconbiosystems.com

Michaela Cesakova
Institute of reproductive medicine and genetics
m.cesakova@ivf-kv.cz

Jennifer Chew
Bio-rad spol. s r.o.
jennifer_chew@bio-rad.com

Luis Enrique Cortes Hernandez
Montpellier Medical University
luis.e.cortes.hdz@gmail.com

Bruno Costa-Silva
Champalimaud Foundation
bruno.costa-silva@research.fchampalimaud.org

Silvia D'Ambrosi
Vrije Universiteit Amsterdam - VUmc
silvia.dambrosi9@gmail.com

Helene Darville
Institut de Recherches Servier
helene.darville@servier.com

Dries De Maeyer
Janssen
ddemaey1@its.jnj.com

Dana Dlouha
IKEM
dadl@ikem.cz

Soheila Dolatabadi
Gothenburg university
soheila.dolatabadi@gu.se

Scott Donovan
Moderna
scott.donovan@modernatx.com

Michaela Dostalova Merkerova
Institute of Hematology and Blood Transfusion
michaela.merkerova@uhkt.cz

Norman Dovichi
University of Notre Dame
ndovichi@nd.edu

Amin El-Heliebi
Medical University Graz
amin.elheliebi@medunigraz.at

Julia Elzanowska
Champalimaud Foundation
julia.elzanowska@gmail.com

Christer Ericsson
Karolinska Institute
Christer.ericsson@ki.se

Pontus Eriksson
Lund University
pontus.eriksson@med.lu.se

Helmut Ernstberger
PerkinElmer
helmut.ernstberger@perkinelmer.com

Zahra Eslami Samarin
Montpellier Medical University
z.eslamisamarin@gmail.com

Anna Fabisiewicz
Maria Skłodowska-Curie Institute - Oncology Center
anna.fabisiewicz@coi.pl

Claudia Felici
University of Bari
claudiafelici85@live.it

Mafalda Ferreira
Cancer Center Amsterdam
mafalda.antunesferreira@gmail.com

Dominik Filipp
Institute of Molecular Genetics, AS CR
dominik.filipp@img.cas.cz

Martyna Filipska
Hosp Univ Germans Trias I Pujol
mfilipska@igtp.cat

Filip Franko
Institute of Biotechnology CAS, v. v. i.
filip.franko@ibt.cas.cz

Marius Gaigalas
UAB Linealibera
m.gaigalas@linealibera.lt

Jean-Pierre Galizzi
Institut de Recherches Servier
jean-pierre.galizzi@servier.com

Marianna Garonzi
Menarini Silicon Biosystems
mgaronzi@siliconbiosystems.com

Stavros Giannoukakos
University of Granada (UGR)
s.p.giannoukakos@hotmail.com

Zoraide Granchi
GenomeScan
order@genomescan.nl

Ewa Grzybowska
Cancer Center Institute
ewag@coi.waw.pl

Ellen Heitzer
Medical University of Graz
ellen.heitzer@medunigraz.at

Markéta Hejníková
Biology Centre CAS
hejnikova@entu.cas.cz

Martin Hemberg
Wellcome Sanger Institute
martin.hemberg@gmail.com

Ana Hernandez de Sande
University of Eastern Finland
ahdesande@gmail.com

Viktor Hlaváč
National Institute of Public Health
viktor.hlavac@szu.cz

Petra Hrubá
IKEM
petra.hruba@ikem.cz

Yuan-Ping Huang
Lam Research
yuan-ping.huang@lamresearch.com

Sarah Hücker
Fraunhofer ITEM
sarah.huecker@item.fraunhofer.de

Petr Hurych
SPADIA LAB, a.s.
petr.hurych@spadia.cz

Jana Jaklova Dytrtova
Institute of Organic chemistry and Biochemistry v.v.i.
dytrtova@uochb.cas.cz

Julia Jarrells
Max-Planck-Institute of Molecular Cell Biology and Genetics
jarrells@mpi-cbg.de

Daliya Kancheva
Janssen Pharmaceutica
dkanchev@its.jnj.com

Žana Kapustina
Thermo Fisher Scientific Baltics
zana.kapustina@thermofisher.com

Veronika Kasparova
Institute of Biotechnology CAS, v. v. i.
veronika.kasparova@ibt.cas.cz

Karel Kleparnik
Institute of Analytical Chemistry of the Czech Academy of Sciences
klep@iach.cz

Vera Kloten
Bayer AG
vera.kloten@bayer.com

Michal Kolář
Institute of Molecular Genetics ASCR
kolarmi@img.cas.cz

Katarina Kolostova
Faculty Hospital Kralovske Vinohrady
katarina.kolostova@gmail.com

Nils Körber
MMI GmbH - Microscopic Single Cell Isolation
koerber@molecular-machines.com

Martin Kovář
Charles University in Prague
martin.kovar@img.cas.cz

Helena Kovaříková
University Hospital Hradec Králové
kovarikovahe@lfhk.cuni.cz

Zdenek Krejcik
Institute of Hematology and Blood Transusion
zdenek.krejcik@uhkt.cz

Vessela Kristensen
Akershus University
v.n.kristensen@medisin.uio.no

Maja Križnik
National Institute of Biology
maja.kriznik@nib.si

Martina Kujanová
SEQme, s.r.o.
martina.kujanova@seqme.eu

Francisca Lameiras
Karolinska Institute
francisca.lameiras@ki.se

Lucie Langerova
Institute of Biotechnology CAS, v. v. i.
lucie.langerova@ibt.cas.cz

Evi Lianidou
National and Kapodistrian University of Athens
lianidou@chem.uoa.gr

Maria Liivrand
University of Eastern Finland
maria.liivrand@uef.fi

Julia Lipecki
University of Warwick
j.lipecki@warwick.ac.uk

Domenica Lovero
University of Bari Aldo Moro
dom.lovero@gmail.com

Francesco Mannavola
University of Bari "Aldo Moro"
francesco.mannavola@gmail.com

Marisa Mariani
Roche
marisa.mariani@roche.com

Alice Mášová
TATAA Biocenter
alice.masova@tataa.com

Paulius Mielinis
Thermo Fisher Scientific
paulius.mielinis@thermofisher.com

Ravindra Naraine
Institute of Biotechnology CAS, v. v. i.
Ravindra.Naraine@ibt.cas.cz

Pavel Ostašov
Charles University
ostasov@volny.cz

Sascha Ott
University of Warwick
S.Ott@warwick.ac.uk

Klaus Pantel
University Medical Center Hamburg-Eppendorf
pantel@uke.de

Adam Pavlinek
University of Cambridge
ap2025@cam.ac.uk

Carlos Pedraz
Hosp Univ Germans Trias I Pujol
cpedraz@igtp.cat

Luca Peruzza
University of Southampton
lp6c17@soton.ac.uk

Nathalie Peynot
INRA
nathalie.peynot@inra.fr

Michael Pfaffl
Technical University of Munich
michael.pfaffl@wzw.tum.de

Pamela Pinzani
University of Florence
p.pinzani@dfc.unifi.it

Nicolas Potie
University of Granada (UGR)
nicolas.potie@ulb.ac.be

Thomas Poyot
IRBA
thomas.poyot@intradef.gouv.fr

Tereza Rašpličková
2nd Faculty of Medicine, Charles University and Motol University Hospital
tereza.rasplickova@fnmotol.cz

Eva Rohlova
Institute of Biotechnology CAS, v.v.i.
eva.rohlova@ibt.cas.cz

Rasa Sabaliauskaite
National cancer institute
rasa.sabaliauskaite@nvi.lt

Timur Samatov
Evotec International GmbH
timur.samatov@evotec.com

Ilaria Santeramo
Cell and Gene Therapy Catapult
ilaria.santeramo@ct.catapult.org.uk

Angela Sanzone
NIBSC
pia.sanzone@nibsc.org

Karina Savvulidi Vargova
Charles University
karin.vargova@gmail.com

Thomas Schlange
Bayer AG
thomas.schlange@bayer.com

Marie Christine Semira
Champalimaud Foundation
mcsemira.md@gmail.com

Vaida Šeputienė
Thermo Fisher Scientific
vaida.seputiene@thermofisher.com

Hamid Sharifi
Karolinska Institutet
hamid.sharifi@ki.se

Miki Shavit
Institute of Biotechnology CAS, v. v. i.
Miki.Shavit@ibt.cas.cz

Radek Sindelka
Institute of Biotechnology CAS, v. v. i.
sindelka@ibt.cas.cz

Remigijus Skirgaila
Thermo Fisher Scientific
remigijus.skirgaila@thermofisher.com

Josef Škoda
Charles University, Faculty of Pharmacy
skodajo@faf.cuni.cz

Agnieszka Śmieszek
Wrocław University of Environmental and Life Sciences
agnieszka.smieszek@upwr.edu.pl

Ondrej Smolik
Institute of Biotechnology CAS, v. v. i.
ondrej.smolik@ibt.cas.cz

Anders Ståhlberg
University of Gothenburg
anders.stahlberg@gu.se

Annika Stechemesser
University of Warwick
a.stechemesser@warwick.ac.uk

Dimitra Stergiopoulou
University of Athens
dimitrastergiopoulou@yahoo.com

Nikolas Stoecklein
Medical Faculty and University Hospital of the Heinrich-Heine-University Düsseldorf
Nikolas.Stoecklein@uni-duesseldorf.de

David Svec
Institute of Biotechnology CAS, v. v. i.
davidsvecu@seznam.cz

Alexey Syutkin
BIOCAD
kartavenko@biocad.ru

Małgorzata Szostakowska
The Maria Sklodowska-Curie Cancer Center and Institute of Oncology
g.szostakowska@gmail.com

Fuchou Tang
Peking University
tangfuchou@pku.edu.cn

Leon Terstappen
University of Twente
l.w.m.m.terstappen@utwente.nl

Jana-Aletta Thiele
Biomedicínské centrum Lékařské fakulty UK v Plzni
jt_150@usc.edu

Justyna Trynda
Wrocław University of Environmental and Life Sciences
justyna.trynda@upwr.edu.pl

Pyotr Tyurin-Kuzmin
M.V. Lomonosov Moscow State University
tyurinkuzmin.p@gmail.com

Amalia Udriste
USAMV
amaliaudriste@gmail.com

Radka Vaclavikova
National Institute of Public Health
radka.vaclavikova@szu.cz

Petra Vainio
TATAA Biocenter AB
petra.vainio@tataa.com

Lukas Valihrach
Institute of Biotechnology CAS, v. v. i.
lukas.valihrach@ibt.cas.cz

Murali Venkatesan
Lam Research Corporation
murali.venkatesan@lamresearch.com

Matous Voboril
Institute of Molecular genetics AS CR
matous.voboril@img.cas.cz

Zuzana Vojtěchová
Faculty of Science, Charles University in Prague
zuzana.vojtechova@natur.cuni.cz

Pavla Vyhlidalova
Palacky University Olomouc
vyhlidalova.pavla@gmail.com

Daniel Žucha
Institute of Biotechnology CAS, v. v. i.
daniel.zucha@gmail.com

Catherine Alix-Panabières
Université de Montpellier
c-panabieres@chu-montpellier.fr

Pavel Burda
Institute of Hematology and Blood Transfusion
barbora.kinska@uhkt.cz

Lia Burkhardt
10x Genomics
Lia.Burkhardt@10xgenomics.com

Laure Cayrefourcq
University of Montpellier
l-cayrefourcq@chu-montpellier.fr

Nikola Curik
Institute of Hematology and Blood Transfusion
barbora.kinska@uhkt.cz

James Downs
Schott Nexterion
james.downs@schott.com

Amin Forootan
MultiD
amin.forootan@multid.se

Diogo Fortunato
Exosomics Siena SpA
dmlfortunato@gmail.com

Andrei Herdean
TATAA Biocenter AB
andrei.herdean@tataa.com

Ondřej Holeňa
ThermoFisher Scientific
Ondrej.Holena@thermofisher.com

Dominika Kadlčíková
Institute of Biotechnology CAS, v.v.i.
dominika.kadlcikova@gmail.com

Peter Karlberg
CelSee
Peter.Karlberg@celsee.com

Imrich Kiss
Fakultní nemocnice Královské Vinohrady Praha
imrich.kiss@uvn.cz

Eva Králová
Beckman Coulter Česká republika s.r.o.
ekralova@beckman.com

Daniel Kraus
Institute of Biotechnology CAS, v.v.i.
dankraus111@gmail.com

Mikael Kubista
Institute of Biotechnology CAS, v. v. i.
Mikael.Kubista@ibt.cas.cz

Maria Lennerås
TATAA Biocenter AB
maria.sjoberg@tataa.com

Chengxun Liu
imec
chengxun.liu@imec.be

Katerina Machova Polakova
Institute of Hematology and Blood Transfusion
barbora.kinska@uhkt.cz

Ondřej Malý
Fakultní nemocnice Královské Vinohrady Praha
ondmaly@gmail.com

Carlotta Mazzitelli
University of Genoa
carlotta.mazzitelli@gmail.com

Paul Oakley
Dolomite Bio
paul.oakley@blacktrace.com

Anup Parikh
Mission Bio
parikh@missionbio.com

Engelbert Precht
Mission Bio
precht@missionbio.com

Chiara Reggio
10x Genomics
Chiara.Reggio@10xgenomics.com

Nezar Rghei
Norgen Biotek Corp.
nezar.rghei@norgenbiotek.com

Marianna Romžová
CEITEC MU
marianna.romzova@ceitec.muni.cz

Rudi Schlaefi
10x Genomics
Rudi.Schlaefi@10xgenomics.com

Naftalie Senderovich
OneCell Medical
naftaliesen@onecellmedical.com

Ingrid Šišková
ThermoFisher Scientific
ingrid.siskova@thermofisher.com

Jiří Smutný
BioVendor – Laboratorní medicína a.s.
smutny@biovendor.cz

Oliver Stephan
Menarini Silicon Biosystems
ostephan@siliconbiosystems.com

Christoph Szymoniuk
Menarini Diagnostic
cszymoniuk@menarini-diagnostics.at

Klaus Tangsgaard
Kem-En-Tec
kn@kem-en-tec.com

Xavier Tekpli
Oslo University Hospital
xavier.tekpli@medisin.uio.no

Pavel Vodička
ÚEM
pvodicka@biomed.cas.cz

Marc H. Wadsworth II
MIT - IMES - Shalek Lab
wadswomh@mit.edu

Rita Zamarchi
IOV-IRCCS
rita.zamarchi@unipd.it

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