Dear Guests and Participants:

Welcome to the 24th Annual Conference of the DGfZ e.V., the society’s second meeting in beautiful Dresden city at the Center for Regenerative Therapies (CRTD).

It is our pleasure to meet with you for inspiring discussions stimulated by this year’s scientific program entitled “Cytometry Across Disciplines 2”. The sessions are again dedicated to an attractive range of hot topics in biomedicine, biotechnology and ecology which essentially benefit from state-of-the-art cytometric tools and emerging technologies for sophisticated, rapid and highly sensitive monitoring and analysis of cells as will be highlighted.

Please be encouraged to visit the interesting accompanying Industrial Exhibition of the DGfZ 2014. The generous and essential support by sponsors and exhibitors is particularly emphasized!

We also invite you to join us for the interactive Welcome Reception on Wednesday evening as well as an entertaining and enjoyable Conference Dinner on the river Elbe steamboat “Gräfin Cosel” on Thursday night.

Wishing you a … multicolor, … multiparameter, … … multipotent, … multidisciplinary … … and MULTI-EXCITING meeting.

Your local organizers

Leoni A. Kunz-Schughart
(President of the DGfZ e.V.)

Denis Corbeil
Organizers:
Leoni A. Kunz-Schughart
Denis Corbeil

Local Organizing Committee:
Edgar Büttner
Theresa Käubler
Mirjam Ingargiola
Melanie Hüther
Alexander Krüger
Franziska Hübner
Carl Gustav Carus Management GmbH

Scientific Advisory Board:
Wolfgang Beisker, München
Lars Blank, Aachen
Hyun-Dong Chang, Berlin
Elmar Endl, Bonn
Wolfgang Fritsche, Jena
Christin Koch, Leipzig
Thomas Kroneis, Graz
Susann Müller, Leipzig
Gabriele Multhoff, München
Frank Alexander Schildberg, Bonn
Stephan Schmid, Regensburg
Frank Schmidt, Greifswald
Torsten Viergutz, Rostock

Local Assistance:
Friederike Manig
Loreen Lehmann
Jana Karbanova
Kristina Thamm
Doreen Reichert
Denise Theil
Wilasinee Chainonthee
Nagwa El Refei Sorour
Franziska Hebenstreit
Vicente Bermudez
Noreen Hinrichs
Thiemo Dinger
Leander Plato
Teodor Todorov
The support from our Home and Host Institutions as well as the following Foundations is highly appreciated:

- Hertie-Stiftung
- DFG SFB 655 Dresden
- ISAC International Society for Advancement of Cytometry
- CRTD Center for Regenerative Therapies Dresden
- Biotec Biotechnology Center TU Dresden
- MEDIZINISCHE FAKULTÄT TECHNISCHE UNIVERSITÄT DRESDEN
- OncoRay National Center for Radiation Research in Oncology Dresden

!!! Thank
We are most grateful to our Sponsors and Exhibitors.
Sessions

Social Events

Poster Sessions & Breaks

DGfZ Members
### Wednesday - 15/Oct/2014

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<td>WELCOME</td>
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<td>12:40 - 02:10 pm</td>
<td>SESSION 1: Cell Imaging Tools for Regeneration</td>
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<td>02:10 - 03:40 pm</td>
<td>SESSION 2: Beyond the Boundaries of Cellular Heterogeneity</td>
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<td>03:40 - 04:10 pm</td>
<td>Coffee Break</td>
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<td>04:10 - 05:40 pm</td>
<td>SESSION 3: Imaging Cell Communication and Interactions</td>
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<td>05:40 - 07:00 pm</td>
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<td>07:00 - 10:00 pm</td>
<td>SESSION 4: Cytometry Discussion Forum</td>
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<td>08:00 - 12:00 pm</td>
<td>CONFERENCE DINNER</td>
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### Friday - 17/Oct/2014

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<td>FAREWELL</td>
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<td>Time</td>
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| 12:30 - 12:40 pm | **WELCOME** - 24th Annual Conference of the DGfZ  
"Cytometry Across Disciplines 2"  
Leoni A. Kunz-Schughart, Dresden, Germany |                                                                                      |
| 12:40 - 02:10 pm| **SESSION 1: Cell Imaging Tools for Regeneration**  
Chair: Elly Tanaka, Dresden, Germany  
Chair: Leoni A. Kunz-Schughart, Dresden, Germany |  
A Dynamic Extracellular Matrix Instructs Regenerative Cell Behavior  
Hans-Georg Simon (Chicago, IL, USA), Sarah Calve, Sarah Mercer  
Resolving the Molecular Behavior of Different Cell Types During Limb Regeneration  
Elly Tanaka (Dresden, Germany)  
Isolation and Gene Expression Profiling of Distinct Neural Progenitor Types in the Mouse and Human Developing Neocortex  
Marta Florio (Dresden, Germany), Mareike Albert, Holger Brandl, Eric Lewitus, Kay Pruefer, Takashi Namba, Elena Taverna, Fong Kuan Wong, Enrico Perini, Alex Sykes, Ina Nüsslein, Barbara Höber, Sylvia Klemroth, Andreas Dahl, Robert Lachmann, Svante Pääbo, Wieland B. Huttner |
| 02:10 - 03:40 pm| **SESSION 2: Beyond the Boundaries of Cellular Heterogeneity**  
Chair: Anja Hauser, Berlin, Germany  
Chair: Denis Corbeil, Dresden, Germany |  
Multi-Epitope Ligand Cartography: Principles and Applications of a Space-Resolved Multichannel Analysis Technique  
Lars Philipsen (Magdeburg, Germany), Peter Reichardt, Burkhart Schraven, Andreas J. Müller  
Hepatic Stromal Cells Induce Myeloid Derived Suppressor Cells From Peripheral Blood Monocytes  
Frank A. Schildberg (Boston, MA, USA), Bastian Höchst, Christian Kurts, Percy A. Knolle, Linda Diehl  
Analysis of Plasma Cell Survival Niches in the Bone Marrow  
Sandra Zehentmeier (Berlin, Germany), Katrin Roth, Zoltan Cseresnyes, Özen Sercan, Raluca Niesner, Hyun-Dong Chang, Andreas Radbruch, Anja Hauser  
Real-Time Deformability Cytometry: High-Throughput Mechanical Phenotyping for Marker-Free Cell Functional Assays  
Jochen Guck (Dresden, Germany), Oliver Otto, Philipp Rosendahl, Alexander Mietke, Stefan Golfier, Angela Jacobi, Christoph Herold, Nicole Töpfner |
| 03:40 - 04:10 pm| **Coffee Break** |                                                                      |
### SESSION 3: Imaging Cell Communication and Interactions

**Chair:** Nils Cordes, Dresden, Germany  
**Chair:** Thomas E. Schmid, München, Germany

- **CD44 and CD44 Variant Isoform v6 as a Cancer and Leukemia Initiating Cell Biomarker**  
  **Margot Zöller** (Heidelberg, Germany)

- **Intercellular Communication Between Bone Marrow-Derived Stem Cells**  
  **Denis Corbeil** (Dresden, Germany), Doreen Reichert, Nicola Bauer, Martin Bornhäuser

- **Cell Imaging With Multi-Spectral Optoacoustic Tomography**  
  **Stratis Tzoumas** (München, Germany), Vasilis Ntziachristos

- **Memory CD8⁺ T Cells Colocalize to IL-7⁺ Stromal Cells in Bone Marrow and Rest in Terms of Proliferation and Transcription**  
  Özen Sercan Alp (Berlin, Germany), Sibel Durlanik, Joachim Grün, Mairi McGrath, Daniel Schulz, Marcus Bardua, Koichi Ikuta, Fritz Melchers, Rene Riedel, Sandra Zehentmeier, Anja E. Hauser, Koji Tokoyoda, Hyun-Dong Chang, Andreas Thiel, Andreas Radbruch

### WELCOME RECEPTION

### SESSION 4: Cytometry Discussion Forum

**Chairs:** Core Manager Committee

- **Report:** Core Facility Management Workshop  
  **Core Manager Committee**

- **Mission, Vision and Portfolio - Round of Introduction of Exhibitors**  
  **Exhibitors**

- **Chipcytometry - a New Versatile High-Content Image Cytometry Platform Enabling Exciting Applications**  
  **Christian Hennig** (Hannover, Germany)
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| 09:00 - 10:30 am | **SESSION 5: Cytometry - From Cancer to Therapy**  
Chair: Martin Bornhäuser, Dresden, Germany  
Chair: Frank Winkler, Heidelberg, Germany | Individualized ErbB-Receptor Targeting of Breast Cancer  
Gero Brockhoff (Regensburg, Germany)  
Monitoring Cell-Based Therapies in Hematology and Oncology  
Martin Bornhäuser (Dresden, Germany)  
Dynamic Intravital Microscopy of Brain Cancer Progression and Response to Therapies: Understanding the Impact of Cell-Cell and Cell-Blood Vessel Interactions  
Frank Winkler (Heidelberg, Germany) |
| 10:30 - 10:50 am | Coffee Break                     |                                           |
| 10:50 - 12:20 am | **SESSION 6: Emerging Technologies**  
Chair: Wolfgang Beisker, München, Germany  
Chair: Ingo Röder, Dresden, Germany | Spectral Analysis in Flow Cytometry  
Mark Dessing (Sony Biotechnology Inc, Germany)  
Label-Free Cell Analyzing Tool to Identify Cell Fate and to Characterize Subpopulations of Sorted Cells  
Karin Schütze (CellTool GmbH, Germany), Steffen Koch, Pjotr Religa, Rainer Gangnus  
A New Approach to Resolve Heterogeneity in Solid Tumors by Digital Image-Based Cell Sorting Using DEPArray Technology Followed by Next Generation Sequencing (NGS)  
Hans Peter Arnold (Silicon Biosystems, Germany)  
Aerosols Revisited: Biosafety Regulations in Europe and Their Impact on Commercial Flow Sorters  
Jens Fleischer (BD Biosciences, Germany)  
The Next Generation in Acoustic Cytometry: Expanded Assays with Acoustic Focusing  
Björn Biedermann (Thermo Fisher Scientific, Germany)  
Adherent Imaging Cytometry Complementing Flow Cytometric Measurements to Address the Diverse Needs of a Research Environment  
Romina Wiedmann (Cenibra GmbH, Germany) |
<p>| 12:20 - 01:05 pm | <strong>POSTER SESSION I</strong>             |                                           |
| 12:20 - 01:40 pm | Lunch                            |                                           |
| 01:05 - 01:50 pm | <strong>POSTER SESSION II</strong>            |                                           |</p>
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| 01:50 - 03:20 pm | **SESSION 7: Single Cell Analysis**  
Chair: Thomas Kroneis, Graz, Austria  
Chair: Vladimir Todorov, Dresden, Germany |
|              | **Taking Expression Profiling to New Dimensions**  
*Mikael Kubista* (Prague, Czech Republic) |
|              | **Next Generation Tissue Profiling**  
*Ola Söderberg* (Uppsala, Sweden) |
|              | **Heterogeneity of Brain Metastases Derived from Melanoma**  
*Beate Rinner* (Graz, Austria), Katharina Meditz, Marie-Therese Frisch, Sabrina Riedl, Helmut Schaider, Bernadette Liegl-Atzwanger, Karl Lohner, Dagmar Zweytick |
|              | **Picoliter Bioreactors: Learning from Single Cells about Large-Scale Bioprocesses**  
*Alexander Grünberger* (Jülich, Germany), *Christopher Probst, Wolfgang Wiechert, Dietrich Kohlheyer* |
| 03:20 - 03:50 pm | **Coffee Break & Poster Viewing** |
| 03:50 - 05:20 pm | **SESSION 8: Analysis of Big Data in Cytometry**  
Chair: Frank Schmidt, Greifswald, Germany;  
Chair: Christin Koch, Leipzig, Germany |
|              | **Analysing High-Throughput, Microscopy-Based Cellular Screens**  
*Lars Kaderali* (Dresden, Germany) |
|              | **Visualization Techniques for Big Data**  
*Jörg Bernhardt* (Greifswald, Germany) |
|              | **Bioinformatical Approaches for the Analysis of Complex Microbial Communities**  
*Joachim Schumann* (Leipzig, Germany), Christin Koch, Susanne Günther, Ingo Fetzer, Susann Müller |
|              | **Human Memory T Cells From the Bone Marrow are Resting and Maintain Long-Lasting Memory**  
*Anna Okhrimenko* (Berlin, Germany), Joachim R. Grün, Kerstin Westendorf, Zhuo Fang, Simon Reinke, Philipp von Roth, Georgi Wassilew, Anja A. Kühl, Robert Kudernatsch, Sonya Demski, Carmen Scheibenbogen, Koji Tokoyoda, Mairi A. McGrath, Martin Raftery, Günther Schönrich, Alessandro Serra, Hyun-Dong Chang, Andreas Radbruch, Jun Dong |
<p>| 05:20 - 05:30 pm | <strong>Coffee Break</strong> |
| 05:30 - 07:00 pm | <strong>Meeting of the DGfZ Members</strong> |
| 08:00 - 12:00 pm | <strong>CONFERENCE DINNER</strong> |</p>
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<td>Chair: Wolfgang Fritzsche, Jena, Germany, Chair: Ulrike Taylor, Mariensee, Germany</td>
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<td><strong>Nano-Sized Drug Delivery Systems for Nucleic Acids</strong></td>
<td>Miriam Breunig (Regensburg, Germany), Luise Tomasetti, Eva-Christina Wurster, Renate Liebl, Achim Goepferich</td>
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<td><strong>Diagnostic Nanoparticle Targeting of the EGF-Receptor Using Single-Domain Antibodies</strong></td>
<td>Kristof Zarschler (Rossendorf, Germany), Kanlaya Prapainop, Eugene Mahon, Louise Rocks, Mattia Bramini, Phil M. Kelly, Holger Stephan, Kenneth A. Dawson</td>
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<td></td>
<td><strong>Introduction to a Multidisciplinary Approach for the Safe Implementation of Nanotechnology in the Environment</strong></td>
<td>Lutz Mädler (Bremen, Germany)</td>
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<td>SESSION 10: Functional Heterogeneity in Microbial Processes</td>
<td>Chair: Susann Müller, Leipzig, Germany, Chair: Christian Dusny, Dortmund, German</td>
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<td><strong>Quantitative Physiology of Single Cells for Linking Phenotype and Environment</strong></td>
<td>Christian Dusny (Dortmund, Germany), Alexander Grünberg, Katrin Rosenthal, Wolfgang Wiechert, Andreas Schmid</td>
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<td><strong>Plasmid Copy Number Variation in Pseudomonas putida Analyzed by Cell Sorting and Digital Droplet PCR</strong></td>
<td>Michael Jahn (Leipzig, Germany), Carsten Vorpahl, Dominique Türkowsky, Martin Lindmeyer, Bruno Bühler, Hauke Harms, Susann Müller</td>
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<td><strong>Flow Cytometry for Energy Balances of Phytoplankton Organisms</strong></td>
<td>Susanne Dunker (Leipzig, Germany), Torsten Jakob, Christian Wilhelm</td>
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<td><strong>Enhanced Viability of Microalgal Populations by Photoperiodic Cycles</strong></td>
<td>Felix Krujatz (Dresden, Germany), Thomas Bley, Jost Weber</td>
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<td><strong>Staphylococcus aureus Infection Induces Human T Cell Apoptosis and Increased Mortality in Humanized Mice</strong></td>
<td>Anja Kathrin Wege (Regensburg, Germany), Janin Knop, Frank Hanses, Nancie Archin, Joachim Gläsner, Andre Gessner</td>
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<td>Lunch Break</td>
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<td><strong>SESSION 11: Klaus-Goerttler-Session - Immunology</strong></td>
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<td>Chair: Petra Bacher, Berlin, Germany</td>
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<td>Chair: Gergely Toldi, Budapest, Hungary</td>
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<td></td>
<td>Detection of Extracellular Vesicles in Inflammation</td>
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<td>Edit Buzás (Budapest, Hungary)</td>
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<td>Monocytes as Biosensors of Type I Interferon Responses in Autoimmunity and Viral Infection</td>
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<td>Andreas Grützkau (Berlin, Germany)</td>
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<td>02:00 - 03:00 pm</td>
<td><strong>SESSION 12: Monitoring Immune Responses</strong></td>
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<td>Chair: Hyun-Dong Chang, Berlin, Germany</td>
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<td>Chair: Axel Roers, Dresden, Germany</td>
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<td>Cytometric Analysis of Antigen-Specific T helper Cell Responses as Diagnostic Sensors in Infection and Allergy</td>
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<td>Alexander Scheffold (Berlin, Germany)</td>
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<td>Single T Cell Fate Tracking and its Implications for Immunotherapy</td>
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<td>Dirk Busch (München, Germany)</td>
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<td><strong>SESSION 13: Meet the Expert Lecture</strong></td>
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<td>Chair: Leoni A. Kunz-Schughart, Dresden, Germany</td>
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<td>Chair: Andreas Deutsch, Dresden, Germany</td>
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<td>The Feasibility of Using Ultrasound for Transcranial Imaging and Targeted Drug Delivery in the Brain</td>
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<td>Kullervo Hynyen (Toronto, Canada)</td>
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<td>04:00 - 04:15 pm</td>
<td><strong>FAREWELL</strong></td>
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Notes:
CONGRATULATIONS
to our 2014 Klaus-Goerttler Awardees

Mrs. Anna Okhrimenko, M.Sc.
Cell Biology, Deutsches Rheuma-Forschungszentrum (DRFZ), Berlin, Germany

Dipl. Ing. Alexander Grünberger
Institute of Bio- and Geosciences (IBG-1)
Forschungszentrum Jülich GmbH, Jülich, Germany

for their excellent scientific work published in internationally recognized journals and highlighted on the Award Certificates (see next pages)

Okhrimenko A et al. Human memory T cells from the bone marrow are resting and maintain long-lasting systemic memory. Proc Natl Acad Sci USA 111(25):9229-34, 2014


Dahlmann M, Okhrimenko A, et al. RAGE mediates S100A4-induced cell motility via MAPK/ERK and hypoxia signaling and is a prognostic biomarker for human colorectal cancer metastasis. Oncotarget. 30;5(10):3220-33, 2014


Klaus Goerttler Award

2014

awarded to

Mrs. Anna Okhrimenko, M.Sc.

at the
24th Annual Conference of the
German Society for Cytometry

for an excellent scientific work, in particular her systematic study on function and specificity of human bone marrow memory T helper cells which substantiates the new concept of resting systemic T cell memory preferentially located in the bone marrow.

Dresden, Oct. 16th 2014

L. A. Kunz-Schughart
(President of the DGFZ e.V.)
Klaus Goerttler Award
2014
awarded to
Dipl.Ing. Alexander Grünberger
at the
24th Annual Conference of the
German Society for Cytometry

for an excellent scientific work, in particular his contribution to the establishment of a novel type of microfluidic single-cell culture system and its first application in fundamental microbiology and applied biotechnology.

Dresden, Oct. 16th 2014

L. A. Kunz-Schughart
(President of the DGFZ e.V.)
Notes:
SESSION 1

Cell Imaging Tools for Regeneration

Wednesday - 15/Oct/2014
12:40 - 02:10 pm
Chairs:

Elly Tanaka, Dresden, Germany
Leoni A. Kunz-Schughart, Dresden, Germany

Deciphering the mechanisms of regeneration, i.e. reactivation of developmental programs to restore damaged or missing tissues and/or organs, is one of the most exciting challenges in modern medicine. Somatic stem cells in concert with dedifferentiation and/or transdifferentiation processes of terminally differentiated cells could actively cooperate in tissue replacement during regeneration. For a better dissection of these mechanisms at cellular and molecular levels numerous animal models are used nowadays and often in a comparative manner. \textit{In vivo} imaging techniques that monitor the spatiotemporal regeneration mechanisms are also emerging. The present session will provide an overview on these new tools that have uncovered novel biological facets of mechanisms underlying the development and regeneration across phylogeny.
A Dynamic Extracellular Matrix Instructs Regenerative Cell Behavior

Hans-Georg Simon, Sarah Calve, Sarah Mercer
Department of Pediatrics and Lurie Children’s Hospital of Chicago Research Center, Northwestern University Feinberg School of Medicine, Chicago IL, USA

The ability to functionally repair tissues damaged by disease or injury remains a significant challenge for regenerative medicine. Using the newt, a salamander that naturally regenerates lost structures and injured tissues, my laboratory is investigating the underlying mechanisms that control the differentiated state of the cell and regulate regenerative processes. Using a multi-tissue approach, employing regenerating fore- and hindlimbs, tail, spinal cord, brain, and heart, we identified concerted gene activities indicative of a molecular signature of regeneration. A striking feature shared by the different regenerating tissues was an extensive and dynamic remodeling of the extracellular matrix (ECM) at the wound site. In particular, we find that the collagen and laminin-rich extracellular environment of differentiated tissues and cells is rapidly replaced by a transitional matrix rich in hyaluronic acid, tenascin-C, and fibronectin. Using high resolution 2-D and 3-D micro-CT and confocal imaging on intact tissue as well as time-lapse microscopy with explanted skeletal and cardiac muscle cells as examples, we present evidence that these regeneration-specific ECM components directly influence the generation and behavior of progenitor cells, including proliferation and migration. Collectively, these results provide a novel understanding of tissue regeneration, suggesting that an evolutionarily conserved, regeneration-specific matrix instructs distinct cell activities that direct in a spatial and temporal sequence the re-building of lost or damaged tissues. Thus, the engineering of nature-tested ECMs may provide new strategic opportunities for the enhancement of regenerative responses in mammals.
Resolving the Molecular Behavior of Different Cell Types During Limb Regeneration

Elly Tanaka
Center for Regenerative Therapies Dresden - CRTD, Germany

The axolotl has held the fascination of generations of biologists for its remarkable ability to regenerate limbs. A key cell type to regeneration in axolotl is the connective tissue fibroblast which acquires the ability to form bone. We have developed molecular genetic methodologies to track these cells during regeneration and understand their molecular properties. Recently we have also started to use the CRISPR/Cas9 system to test gene function during regeneration.
The expansion of the neocortex, a major hallmark of primate evolution, requires greater numbers of neurons to be generated, during neocortical development, in the process of neurogenesis. In all mammalian species, neurogenesis is driven by distinct subpopulations of neural progenitor cells (NPCs), each contributing different numbers of neurons to the final cortical cytoarchitecture. The neuron output of a given NPC type is largely determined by the extent of its proliferative potential (i.e. its capacity to self-amplify) and differentiative potential (i.e. its capacity to generate distinct, lineage-wise downstream NPC types and neurons). In this respect, chief interspecies differences have been identified – most notably between primates and rodents – as to the mode of division of specific classes of NPCs. Remarkably, primate NPCs are endowed with greater proliferative and differentiative potentials compared to their rodent counterparts, conceivably reflecting the increased numbers of neurons that characterize the expanded primate neocortex. The molecular mechanisms ultimately responsible for the greater neuron output of primate NPCs, however, remain to be elucidated. In order to investigate this issue, we have established a novel protocol to isolate different NPC types in the mouse and human developing neocortex. This technique is based on a combinatorial code of molecular and morphological cell-type markers, which allows NPCs to be labeled ex-vivo and to be isolated by fluorescent-activated cell sorting. We have carried out transcriptome analysis and compared the gene-expression profiles of the isolated NPC populations within and between species. This has revealed gene expression patterns unique to human NPCs, potentially underpinning their species-specific mode of division and greater neuron output.
SESSION 2

Beyond the Boundaries of Cellular Heterogeneity

Wednesday - 15/Oct/2014
02:10 - 03:40 pm
Immune cells are highly motile and travel throughout the whole body in order to survey and search for pathogens. Therefore, the communication between various cells of the immune system, as well as interactions between immune cells and other, tissue-specific cell types is a key feature for an efficient function of the immune system. Consequently, analyzing the behavior of cells in their genuine tissue context, thereby including the information on cellular localization and interactions is crucial in order to understand the various functions and mechanisms of cell plasticity and response. However, the diversity of the tissue composition with numerous different cellular players is still a challenge with respect to data acquisition on the one hand and data analysis on the other hand. In this session we present novel approaches to analyze cellular behavior within the complex environments of tissues. One focus of the session will be the acquisition of multiplexed data in microscopy. We will also discuss how to measure and quantify cellular localization and interactions in the tissue in an unbiased, automated way. Finally, we will focus on the role of tissue-specific stromal cells in the maintenance as well as the modulation of immune responses.
Multi-Epitope Ligand Cartography: 
Principles and Applications of a Space-
Resolved Multichannel Analysis Technique

Lars Philipsen, Peter Reichardt, Burkhart Schraven, 
Andreas J. Müller
Institue for Molecular and Clinical Immunology, Otto-von-Guericke University of Magdeburg, Germany

The proper function of the immune system requires correct interactions of a variety of different cells at distinct organs. Within these cells, signalling networks control the behaviour of each individual cell in response to its environment. To investigate the complexity of the immune response, we use multi-epitope ligand cartography (MELC), which is able to measure more than 50 parameters with optical resolution on fixed tissue or cell samples. Using this technique, we studied the signalling network in stable immunological synapses (IS) between T cells and antigen-presenting cells by measuring the simultaneous phosphorylation and localization of signalling and structural molecules at the level of individual IS. We could show that following a period of structural enforcement of cell-cell contact between 10 and 30 minutes, an extensive increase in signalling molecules took place at the synapse from 60 to 120 minutes. This "synaptic signalling maturation" occurred in the course of discrete microcluster formation of molecules such as pCD3ζ, pZAP70 and pLAT, which became co-localised in the central synapse. Besides the investigation of subcellular signalling processes, the MELC technology can be applied for characterizing cellular processes in great detail on the tissue level: We can prepare up to four tissue/cell compartments of different immune organs (e.g. lymph node, thymus or spleen) on the same slide for one MELC experiment, which allows us to systemically monitor immune responses of mice and humans not only on the level of frequencies of the different cell populations, but also the their localization within the organs, and to dissect the interactions between these cells.
Myeloid derived suppressor cells (MDSCs) are a heterogeneous population of cells associated with the suppression of immunity. However, little is known about how or where MDSCs are induced and from which cells they originate. Here, we investigated the capacity of human hepatic stellate cells (HSCs) to transform peripheral blood monocytes into MDSCs. Using mature peripheral blood monocytes co-cultured with HSCs, our study showed that only in their activated state could stromal cells convert mature peripheral blood monocytes into MDSCs. As HSCs are activated during chronic inflammation, the subsequent local induction of MDSCs may prevent ensuing excessive liver injury. HSC-induced MDSCs functionally and phenotypically resemble those isolated from liver cancer patients. Thus, our data suggest that local generation of MDSCs by liver-resident HSCs may contribute to immune suppression during inflammation and cancer in the liver.
Analysis of Plasma Cell Survival Niches in the Bone Marrow

Sandra Zehentmeier¹, Katrin Roth¹, Zoltan Cseresnyes¹, Özen Sercan², Raluca Niesner³, Hyun-Dong Chang², Andreas Radbruch², Anja Hauser¹

¹Immunodynamics, Deutsches Rheuma-Forschungszentrum; ²Cell Biology, Deutsches Rheuma-Forschungszentrum; ³Biophysical Analytics, Deutsches Rheuma-Forschungszentrum (DRFZ) Berlin, Germany

Long-term stable antibody titers, provided by memory plasma cells (PC), are key to life-long protective immunity. Residing in the bone marrow (BM), memory PC have been shown to survive for months to years in the absence of antigen, supported by a special microenvironment: the PC survival niche. Various BM cell types have been reported to contribute to this niche by provision of survival factors, amongst them CXCL12-secreting reticular stromal cells and hematopoietic cells like eosinophils and megakaryocytes. In the BM, most cell types of hematopoietic origin show a high turnover, thereby raising the question of how a stable supply of PC survival factors by hematopoietic accessory cells is guaranteed.

The quantitative analysis of niche cell localization within BM tissue provides the basis for a systematic characterization of this multicomponent system. To determine the microanatomical composition of the niche and to gain insight into the turnover of accessory cells and reticular stromal cells, we combined a fluorescent mouse model visualizing stromal cells, an automated image analysis approach for bone histology and in vivo proliferation analysis.

By automated image analysis, we quantified the contacts of BM PC with eosinophils, megakaryocytes, lymphocytes and reticular stromal cells and characterized the neighborhood of PC. To account for varying population frequencies and to test whether the observed colocalization counts could also result from a random tissue distribution of these cells, we developed a simulation tool for random BM cell positioning. Thus, we found that PC are predominantly contacting reticular stromal cells in a non-random fashion. Combined flow cytometric and histological proliferation analysis of stromal cells and eosinophils of the PC niche identified reticular stromal cells as non-proliferative, stable niche component. In support of this, intravital microscopy of the BM revealed that static PC form stable contacts with stromal cells. In contrast, eosinophils in the vicinity of memory PC displayed a high turnover.

These findings support the idea of a stable microenvironment being maintained through constant attraction of fluctuating accessory cells, probably organized by static reticular stromal cells.
Real-Time Deformability Cytometry: High-Throughput Mechanical Phenotyping for Marker-free Cell Functional Assays

Oliver Otto\textsuperscript{1}, Philipp Rosendahl\textsuperscript{1}, Alexander Mietke\textsuperscript{1}, Stefan Golfier\textsuperscript{1}, Angela Jacobi\textsuperscript{1}, Christoph Herold\textsuperscript{1}, Nicole Töpfner\textsuperscript{1}, Jochen Guck\textsuperscript{1,2}
\textsuperscript{1}Biotechnology Center TU Dresden, Germany; \textsuperscript{2}Cavendish Laboratory, University of Cambridge, United Kingdom

Changes in cell function are often accompanied by alterations of the intrinsic cell structure particularly the cytoskeleton. This leads to distinct mechanical changes. For example, cells become softer during malignant transformation and stiffer during differentiation. Exploiting the mechanical phenotype of cells as an inherent, label-free marker requires a high-throughput and robust measurement technique. Here, we introduce real-time deformability cytometry (RT-DC) for mechanical single cell classification of heterogeneous cell populations at rates of several hundred cells per second in real-time. Performing RT-DC on primary human hematopoietic stem cells and mature blood cells we demonstrate its capability to detect lineage and source specific mechanical phenotypes. We also find that different stages of the cell cycle possess a unique mechanical fingerprint allowing the distinction between cells in G2 and M phase. In summary, RT-DC represents a novel flow cytometric approach that enables the translation of mechanical phenotyping from basic research into applications in biology and medicine.
24th Annual Conference of the German Society for Cytometry
October 15th-17th 2014
Center for Regenerative Therapies, Dresden

Session 3
Imaging
Cell Communication
and Interactions

Wednesday - 15/Oct/2014
04:10 - 05:40 pm

CRTD
Biotec
Oncoray
Chairs:

**Nils Cordes**, Dresden, Germany  
**Thomas E. Schmid**, München, Germany

Throughout past years, *in vivo* imaging of cells using fluorescent and radionuclide labeled tracers has greatly improved our knowledge not only in developmental biology but also in the understanding of disease, e.g. in the fields of oncology and cardiovascular dysfunction. Molecular imaging of dynamic cell interactions are key to characterize and measure biological processes on a molecular level.

This session particularly focusses on the imaging of cell-matrix / cell-cell interactions and with the particular aim to gain insight into the use of innovative imaging techniques such as intra-operative imaging, Fluorescence Molecular Tomography (FMT) and Multi-Spectral Opto-acoustic Tomography (MSOT) that can improve the visualization of cell communication processes. We will further discuss the identification and characteristics of molecules of interest in cell-matrix interactions with multiple functions and potential as biomarker and/or targeting molecule for stem cells and/or aggressive cancer cell populations.
CD44 and CD44 Variant Isoform v6 as a Cancer and Leukemia Initiating Cell Biomarker

Margot Zöller
Department of Tumor Cell Biology, University Hospital of Surgery, Heidelberg, Germany

CD44 is an adhesion molecule that varies in size due to glycosylation and insertion of so called variant exon products. The so called CD44 standard isoform (CD44s) is highly expressed in many cells of the organism and most abundantly in cells of the hematopoietic system, whereas expression of CD44 variant isoforms (CD44v) is more restricted. Nonetheless CD44s and CD44v are known as a stem cell marker, first described for hematopoietic stem cells and later for leukemia initiating cells and a wide range of carcinoma. Moreover, CD44 is so far one of the few cancer initiating cell biomarkers. Taking the abundance of CD44 expression and a minor phenotype seen in mice with a targeted deletion of CD44, this is most surprising. Furthermore, there is no evidence for CD44 mutations to account for an oncogenic shift of CD44. CD44 also plays, at least, no dominating role as a transcription factor initiating transcription of oncogens or metastogens. Last to mention, it does not display kinase activity that could account for initiating signaling cascades. Thus, this protein acts rather exclusively by cooperativity at various levels. It associates with receptor tyrosine kinases, integrins, proteases, cytoplasmic signaling molecules and cytoskeleton elements, whereby it mostly affects motility, invasiveness and apoptosis resistance. It is the main receptor for HA and also binds additional matrix proteins. Together with associated integrins and proteases this further supports motility and invasiveness. Via its cellular ligands CD44 triggers activation of signaling cascades in target cells, particularly endothelial cells. I will discuss how by this multitude of interactions CD44 takes over a central role in tumor progression, which is suggested to be mediated by a subpopulation of migrating cancer initiating cells. This includes a focus on metastasis being based on, but independent of oncogenic transformation and rather resembling embryogenesis and repair processes.
Intercellular Communication Between Bone Marrow-derived Stem Cells

Denis Corbeil, Doreen Reichert¹, Nicola Bauer¹, Martin Bornhäuser²
¹Tissue Engineering Laboratories (BIOTEC), Technische Universität Dresden; ²Medizinische Klinik und Poliklinik I, Universitätsklinikum Carl-Gustav Carus Dresden, Germany

Cell-to-cell communication is a crucial prerequisite for the development and maintenance of multicellular organisms. This is particularly true for bone marrow microenvironments, the so-called niches, where distinct cellular components are crosstalking to maintain and support hematopoiesis. In addition to a direct contact between hematopoietic stem and progenitor (S/P) cells with bone marrow-associated mesenchymal stromal cells and soluble factors released by them, other mechanisms of intercellular communication have recently emerged. For our investigations we are using a co-culture system consisting of human CD34⁺ hematopoietic S/P cells growing on primary multipotent mesenchymal stromal cells (MSCs) as a feeder cell layer. With the help of this system, we demonstrated that small membrane vesicles are released by the CD34⁺ hematopoietic S/P cells during their differentiation and are internalized by the MSCs afterwards. This phenomenon was not observed with neighboring hematopoietic S/P cells as potential recipient cells suggesting selectivity of the transfer process. The internalization of exosome-like vesicles by feeder cells is consistent with their function in communication. They not only act as signaling devices by stimulating target cells via cell surface expressed ligand(s) but also by transferring receptor/adhesion molecules or small RNA between cells. Besides the intercellular shuttling of membrane vesicles, we recently examined and characterized the biogenesis of nanotubular-like highway structures between hematopoietic S/P cells as an alternative mechanism of communication. Indeed, hematopoietic S/P cells develop thin actin-dependent plasma membrane protrusions that bridge a distance up to several micrometers (>100). These membranous structures are used to transfer certain components. Thus, the complexity of mechanisms regulating the intercellular communication within the bone marrow niches has grown exponentially in recent years. Further investigations using ex vivo culture systems as well as the development of animal models to demonstrate their impact in vivo are urgently required.
Cell Imaging with Multi-spectral Optoacoustic Tomography

Stratis Tzoumas¹, Vasilis Ntziachristos¹,
¹Institute for Biological and Medical Imaging (IBMI), Technische Universität München and Helmholtz Zentrum München, Neuherberg, Germany

Microscopy plays a critical role in cell biology, enabling observations of cell-cell and cell-host interactions in vivo. However, the limited penetration depth of microscopy methods only allows for superficial observations. Many applications however require high resolution cell imaging at different scales. Optoacoustic tomography, also termed photoacoustic tomography, is a novel imaging modality that offers optical contrast with ultrasound resolution. Optoacoustic imaging measures acoustic waves generated by the absorption of pulsed light in tissue and forms high resolution optical absorption images within several millimeters to centimeters deep inside tissues. Multispectral laser excitation and spectral unmixing methods further allow for resolving light absorbing moieties from the absorbing tissue background, with high sensitivity and specificity. Multi-spectral optoacoustic tomography (MSOT) offers the potential to image in high resolution cells tagged with optical labels. This feature can enable longitudinal cellular biology studies well beyond the depths reached by optical microscopy. Herein we demonstrate the capabilities of MSOT in imaging cells labeled with fluorescent dyes in animals, and assess the sensitivity capabilities of the technology in such applications. We further demonstrate the advantages of MSOT technology over the widely employed diffuse-light imaging methods, and conclude that MSOT may become a method of choice in longitudinal whole body cell imaging studies.
Memory CD8+ T Cells Colocalize to IL-7+ Stromal Cells in Bone Marrow and Rest in Terms of Proliferation and Transcription

Özen Sercan Alp1, Sibel Durlanik2, Joachim Grün1, Maíri McGrath1, Daniel Schulz1, Marcus Bardua1, Koichi Ikuta1, Fritz Melchers3, Rene Riedel1, Sandra Zehentmeier1, Anja E. Hauser1, Koji Tokoyoda1, Hyun-Dong Chang1, Andreas Thiel2, Andreas Radbruch1
1Deutsches Rheuma-Forschungszentrum; 2Charité - Berlin-Brandenburger Center for Regenerative Therapies (BCRT); 3Max Plank Institute for Infection Biology, Berlin, Germany

Memory CD8+ T cells are important components of immune responses against recurrent infections and understanding their biology is crucial to improve their protective capabilities. It remains to be shown how and where memory CD8+ T cells are maintained over long periods of time. Memory CD8+ T cells that were generated upon a specific immune response have been shown to populate in bone marrow (BM); however, their function and persistence have not been thoroughly investigated. In this study we aimed to elucidate whether memory CD8+ T cells reside in distinct niches in BM as resting cells.

Our results show that following peptide immunization or LCMV infection the BM hosts equal, if not greater, numbers of Ag-specific memory CD8+ T cells compared to the spleen indicating that BM is a major location for memory CD8+ T cell maintenance. A substantial number of both Ag-specific and non-specific memory CD8+ T cells in the BM express CD69, also known as “early activation marker”. Despite this, the vast majority of BM cells are resting in terms of proliferation and gene expression suggesting that CD69 marks resident rather than activated cells. Memory CD8+ T cells reside next to BM stromal cells that produce IL-7, which is the major survival factor for mCD8+ T cells. In conclusion, our findings suggest that memory CD8+ T cells reside in BM as resting rather than activated cells and are maintained in distinct survival niches involving IL-7 producing stromal cells.
24th Annual Conference of the German Society for Cytometry

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SESSION 4

Cytometry Discussion Forum

Wednesday - 15/Oct/2014
07:10 - 10:00 pm
Chairs: *Core Manager Committee*
Elmar Endl (Bonn), Hyung-Dong Chang (Berlin),
Desiree Kunkel (Berlin), Toralf Kaiser (Berlin),
Mario Follo (Freiburg), Stoffan Schmitt (Heidelberg),
Ina Schäfer (Mainz), Jens Hartwig (Mainz)

The previous title of our Wednesday late evening session “Core Manager Workshop” has been modified. “Cytometry Discussion Forum” much better reflects the real format of this session. It is supposed to give the participating industry, experienced scientists and also young investigators an open time slot to meet and discuss their analytical needs and challenges in a relaxed and friendly atmosphere. As usual, the session will be chaired by one or two core managers and will spontaneously be adapted according to the inquiries and desires of the DGFZ 2014 attendees. Industry will have the chance to highlight their company’s profile and expertise. Note: A separate core facility manager workshop for experts is scheduled as pre-conference program. Important issues and conclusions from this workshop will be briefly presented in the Cytometry Discussion Forum session by the chairs.
Report: Core Facility Management Workshop

Core Manager Committee

Members of the Core Manager Committee will give a brief resumé about the topics of the pre-conference “Core Facility Management Workshop: Basics & More”.

Mission, Vision and Portfolio -
Round of Introduction of Exhibitors

DGfZ Exhibitors

Companies:
AHF Analysentechnik AG, Germany
Beckman Coulter GmbH, Germany
Becton Dickinson, Germany
Bio-Rad Laboratories, Inc., USA
BioLegend, Germany
Biozol Diagnostica Vertrieb GmbH, Germany
CellTool GmbH, Germany
Cenibra GmbH, Germany
Fluidigm Europe B.V., The Netherlands
IBA GmbH, Germany
Life Technologies GmbH, Germany
Mauna Kea Technologies, France
Merck Millipore, Germany
Miltenyi Biotec GmbH, Germany
Polysciences / Bangs Laboratories, USA
Propel Labs Inc., Germany
Silicon Biosystems, Germany
Sony Biotechnology Inc., Germany
Sysmex Partec GmbH, Germany
TATAA Biocenter AB, Sweden
Chipcytometry - a New Versatile High-Content Image Cytometry Platform Enabling Exciting Applications

Christian Hennig
ZELLKRAFTWERK GmbH, Germany

There is a growing interest in high-content biomarker analysis on a single cell level - like biomarker discovery on tumor stem cells, phenotyping of antigen-specific immune responses or quality control of cell-based therapies. Flow cytometry covers many needs within this research area. However, some applications are impossible or difficult to solve using flow. We have developed Chipcytometry to complement flow cytometry in these areas. We use disposable microfluidic chips to permanently keep live- or PFA-fixed cells or tissue sections on defined positions inside these chips. In this way, each cell can be called individually again and again over a period of at least 16 month, and a virtually unlimited set of markers can be analyzed per cell. Using this Chipcytometry technology platform, together with our customers we developed and published many applications to put cytometry to the next level.

Chipcytometry.discover: This application allows building biobanks of valuable stratified patient samples. Stepwise analysis of a virtually unlimited number of biomarkers without loss of sample helps to discover new biomarkers and cell types much faster than before.

Chipcytometry.rare: This application helps to detect very rare events like CTC or progenitor cells and focus on those cells for in-depth phenotyping with an unlimited marker set.

Chipcytometry.store: The microfluidic chips allow to store and ship your cell/tissue samples under controlled conditions for >16 month without losing cells or biomarkers. If needed, new or additional biomarkers can be analyzed on the exact same cells as the sample is not consumed or lost during analysis.

Chipcytometry.tissue: As an add-on, Chipcytometry allows you to put tissue sections inside chips and analyze tissue composition, infiltration/distribution of immune cell infiltrates or proliferation status in biopsies or tissue sections up to 2x1 cm in size.

Chipcytometry.livecell: The live-cell extension allows to measure Ca-flux, apoptosis, chemokinesis and other live cell assays on a single cell level on thousands of cells in parallel using fluorescent signals as readout - if needed combined with pre/post stimulation phenotyping of these cells.
SESSION 5

Cytometry -
From Cancer to Therapy

Thursday - 16/Oct/2014
09:00 – 10:30 am
Chairs:

**Martin Bornhäuser**, Dresden, Germany
**Frank Winkler**, Heidelberg, Germany

State-of-the-art cytometric devices have at any time throughout the past 50 years taken us another step forward in the understanding of malignant disease. Today, a broad range of tools from easy-handling to sophisticated is applied in basic, pre-clinical and clinical cancer research. In this context, we discussed their usefulness during the latest DGfZ conferences to identify, enrich or isolate and study particular cancer cell subpopulations such as putative cancer stem cells (CSCs) or circulating tumor cells (CTCs). This year’s cancer session is dedicated to the focus of theragnostics covering aspects such as cell-based therapies in haematological malignancies, individualized targeted therapy for patients with solid tumor and treatment monitoring in challenging pre-clinical orthotopic cancer models.
Individualized ErbB-Receptor Targeting of Breast Cancer

Gero Brockhoff
Department of Gynecology and Obstetrics, University of Regensburg, Germany

Anti-ErbB2-receptor targeting with trastuzumab (as a gold standard) became an obligate therapy regimen for Her2-positive, invasively growing breast cancer. However, de novo or acquired resistance occurs in a majority of treated patients. Multiple molecular, cellular, and immunological mechanisms have been attributed to the failure of anti-ErbB2-targeting. Consequently, novel therapeutic antibodies and small molecule tyrosine kinase inhibitors (TKI) have been developed to inhibit ErbB2 and its related receptor tyrosine kinases (RTK). The molecular/cellular mechanisms of action that underlie their application are, however, often insufficiently investigated and consequently incompletely understood. Accordingly, drawing on alternative, target specific strategies is primarily an arbitrary approach.

This presentation gives examples of preclinical ErbB-receptor specific targeting of breast cancer cells with different ErbB-receptor coexpression profiles. In particular, the (potential) impact of individual ErbB-receptors (ErbB1, ErbB3, and ErbB4) on anti-ErbB2-targeting will be discussed. For example, evidence for enhanced anti-ErbB2 treatment efficiency by a complementary ErbB1-targeting will be provided. In contrast, inhibiting anti-ErbB4 might be rather counterproductive. Moreover, a strong growth factor-specific impact on the outcome of receptor targeting with anti-ErbB antibodies and TKIs will be demonstrated.

Overall, cytometric approaches, e.g., dynamic proliferation assessment, enable to quantitatively evaluate the effectiveness of ErbB-directed treatments. Flow cytometric analyses support an evidence based design of modular anti-ErbB targeting whereby treatment efficiency of ErbB2-positive breast cancer might be enhanced.
Cell-based therapies relying on genetic engineering of autologous T cells (Chimeric antigen receptor T cells) are currently developed and clinically tested in patients with lymphoma, leukemia and solid tumors. Nevertheless, allogeneic hematopoietic cell transplantation is still among the most effective cell-based therapies with regard to the efficiency of engraftment and the potency of allogeneic immune effects. Novel developments in cellular therapies beyond donor lymphocyte infusions to treat leukemia relapse have been implemented. The therapeutic armentarium has been broadened to non-hematopoietic mesenchymal stromal cells which may be used as therapeutics for acute GvHD but also as shuttles for gene therapy. Additionally, adoptive immunotherapy with antigen-specific T cells or the aforementioned chimeric-antigen receptor transduced donor T cells (CARTs) have helped to make the antileukemic effects after allogeneic HCT more specific. Finally, pharmacological activation of regulatory T cells as well as their adoptive transfer are currently tested with the aim of inducing tolerance and alleviate GvHD symptoms. In patients with acute leukemia, the adoptive transfer of allogeneic NK cells is applied in order to harness innate immunity against cancer cells. Flow cytometry is the major diagnostic tool to quantify the frequency of adoptively transferred T and NK cells in i) the graft and ii) the patient before and after the infusion. Novel multimer-based FACS assays have been introduced in order to quantify the frequency of antigen-specific T cells. Intracellular staining of transcription factors like Foxp3 are implemented into clinical routine in order to quantify regulatory T cells in cancer patients and cases with graft-versus-host disease. The quantification of these specific cell subsets has gained prognostic and predictive relevance in the recent years.
Dynamic Intravital Microscopy of Brain Cancer Progression and Response to Therapies: Understanding the Impact of Cell-Cell and Cell-Blood Vessel Interactions

Frank Winkler
Neurologische Klinik und Nationales Tumorzentrum, Abteilung Neuroonkologie, Universitätsklinikum Heidelberg, Germany

To successfully grow, invade and metastasize, tumor cells have to closely interact with the microenvironment, including blood vessels. A better understanding of the dynamic and reciprocal nature of this interaction can improve our insight into the mechanisms of tumor dissemination, and tumor angiogenesis. We therefore established novel animal models that allowed us to image cancer cells and their heterogeneity, blood vessels, non-malignant cell types, and their interaction in the live brain in subcellular resolution. For that, intravital imaging with multiphoton microscopy through a chronic cranial window was used to track individual glioma cells, and circulating metastasizing cancer cells in real time over minutes to months. This talk will cover the role of cancer cell heterogeneity, cancer cell crosstalk, pre-existing blood vessels, angiogenesis, and cancer cell dormancy for the single steps of glioma growth, and brain metastasis formation. In addition, novel insights into the mechanisms of action of antitumor therapies will be reported. The unique opportunity to study metastasis and tumor progression as a process rather than an endpoint opens new roads of research. Advanced intravital imaging is especially valuable to pinpoint how different cancer cells behave differently in the same microenvironment, but also how therapies exactly work, and will give clues how they can be optimized in the future. Finally, the development of novel fluorescent reporter systems for gene expression, ion concentrations, cancer cell stemness/quiescence, cellular organelles, and physiological parameters will greatly extend the information we will get about the metastatic cascade and tumor progression from \emph{in vivo} multiphoton microscopy in the future. Some novel applications of these reporter systems will be presented in the talk.

References:
24th Annual Conference of the German Society for Cytometry
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Center for Regenerative Therapies, Dresden

Session 6
Emerging Technologies

Thursday - 16/Oct/2014
10:50 - 12:20 am
Chairs:

**Wolfgang Beisker**, München, Germany  
**Ingo Röder**, TU Dresden, Germany

Today, cytometric technologies are indispensable and integral in many research areas and life science-related disciplines. The development and progress of tools for cell analysis from advanced multicolor flow cytometry via cell sorting strategies to modern life-time imaging and sophisticated microscopic cell and tissue monitoring has always benefited from open-minded, critical discussions and interactions between academia and industry. This session is set up to provide a pleasant environment for the presentation and discussion of new methodological developments and emerging technologies which are supported by the industrial partners of the DGfZ and its members, and are likely to facilitate our future-oriented cell and tissue research.
Spectral Analysis in Flow Cytometry

Mark Dessing
Sony Biotechnology Inc., Germany

Spectral analysis has been the focus of many research projects in flow cytometry over the last decade. In confocal microscopy, spectral detectors have been in use since a long time with the big advantage that the exposure time can be adjusted in order to collect enough photons. In flow cytometry however, the exposure time of a single cell is limited to 3 to 4 microseconds and the amount of photons emitted from bound dyes is considerably less, leading to reduced signal to noise ratios. The SP6800 is the first commercially available flow cytometer that doesn't use conventional PMT's, optical filters and mirrors to quantify the amount of emitted photons from different dyes but instead uses a prism assembly to separate the light according to wavelength and image it on a 32 channel PMT array. This configuration allows the user to measure the full spectrum from 420 nm up to 800 nm for each individual cell and can be used to get precise spectral fingerprints from each individual cell. This spectral information, with the help of advanced un-mixing algorithms, can help identify subpopulations of cells, measure (and subtract) cellular auto-fluorescence or quantify energy transfer. This presentation will focus on the technical considerations leading to the development of a spectral flow cytometer and will show some of its potential in multi-color flow cytometry.
Label-free Cell Analyzing Tool to Identify Cell Fate and to Characterize Subpopulations of Sorted Cells

Karin Schütze¹, Steffen Koch¹, Pjotr Religa², Rainer Gangnus¹
¹CellTool GmbH, Bernried, Germany; ²Karolinska Institute, Department of Medicine, Stockholm, Sweden

In biomedical research and application there is an increasing demand for non-invasive and highly sensitive cell recognition methods. Raman spectroscopy (RS) is an emerging technology with high potential in biomedical applications such as identification of specific cell types or monitoring cell state or differentiation processes.

We used a “biocompatible” Raman microscope system combined with optical tweezers (BioRam® CellTool, Bernried) that allows cell characterization on a single cell level from adherently growing cell cultures or from cells emerged in solution – independently of being alive, fixed or embedded in paraffin. We reliably could identify different cell types, discriminate cell phase and even monitor stem cell differentiation without any labeling such as antibody-based markers or fluorescence molecules. In order to determine differences in the biochemical component patterns, Raman-spectra of cell groups were obtained and analyzed with principal component analysis (PCA) and the support vector machine (SVM). In this work Raman spectroscopy was used to measure cell viability in cell lines SAOS-2 and SW-1353 and to discriminate temperature induced apoptotic and necrotic from healthy cells. Furthermore, we evaluated the potential of Raman spectroscopy to identify subpopulations of murine MSCs cells, labeled with CD44, CD90 and Sca-1 surface markers. Sample preparation for Raman spectroscopy was 4 % PFA to fix the cells. MACS- and FACS-sorted cell populations were analyzed by Raman Spectroscopy and compared with results of gene expression assays.

Results: Temperature induced apoptosis and necrosis was verified by standard methods and compared to the Raman spectroscopic results. SVM analysis showed a discrimination accuracy of 99.7 % for viable and dead cells and 96.3 % for viable, apoptotic and necrotic cells. Raman spectra of labeled MSC subtypes yield a strong overlap in the PCA-plots but there were cells that clearly differ. Gene expression analysis confirmed these findings.

Conclusions: Raman spectroscopy is an exciting tool for non-destructive cell analysis enabling discrimination of cell types, cell state and differentiation or reaction upon drugs and toxins in a highly sensitive and efficient manner.

This project was partly funded by EU 7th Program for research, technological development and demonstration under grant agreement No 279288
A New Approach to Resolve Heterogeneity in Solid Tumors by Digital Image-Based Cell Sorting Using DEPArray Technology Followed by Next Generation Sequencing (NGS)

Hans Peter Arnold
Silicon Biosystems, Germany

Heterogeneity is a big challenge limiting the effectiveness of molecular analysis of solid tumors. The introduction of digital analytical methods, like NGS and digital PCR, has helped manage sample heterogeneity through higher depth of analysis, in terms of minor frequency variant detection. However, the heterogeneity of tumor samples, like formalin fixed paraffin embedded (FFPE) samples, still does not allow to get a clear view of the exact genetics going on in different cell compartments of the tumor, like cancer, stromal, mesenchimal etc. and makes it still difficult to detect CNV with precision and sensitivity.

DEPArray is a new technology capable of manipulating individual cells using a digitally controlled dielectrophoretic field on a semiconductor chip where cell identification is achieved via image based cell sorting. Single circulating tumor cells can be isolated from blood as well as pure pools of tumor or stromal cells from disaggregated FFPE tissue.

In this presentation, we show how the digital, image based sorting of disaggregated FFPE tumor cells allows to simplify the heterogeneity of this type of sample. We present data generated with a NGS cancer gene panel from DNA extracted from two pure pools each of 300 digitally selected tumor and stromal cells vs. DNA directly extracted from the FFPE sample. A comparison of the three data sets clearly demonstrates that DEPArray-based cell sorting allowed the unambiguous assignment of the genetic origin of each variant present in each cell subpopulation, enhances detection of CNV and enables further identification of more subpopulations of cells.
Aerosol generation by cell sorters has been seen for a long time as a fact. Only few studies have actually investigated the real aerosol generation rates and distributions. Recent studies have shown the actual size distributions at various pressures and the aerosol spread around the instrument. European biosafety regulations require aerosol spread control at the different biosafety levels. However, national laws of the European member states often impose even stricter regulations on the research lab. Additionally, the recently published ISAC guidelines for Cell Sorter Biosafety Standards have generated a lot of attention for the biosafety issue. The talk will illustrate the aerosol generation of cell sorters, highlight the biosafety recommendations of the ISAC guidelines and compare them with the European and national laws (Germany and Switzerland) and finally show some technical solutions for commercial instruments. Emphasis will also be on regulatory requirements for testing and certification of biosafety equipment.
The Next Generation in Acoustic Cytometry: Expanded Assays with Acoustic Focusing

Björn Biedermann
Thermo Fisher Scientific, Germany

Acoustic focusing cytometry, introduced in 2005, is a technology that uses ultrasound waves to position cells into a single focused line within a flow cell. The ability to focus cells into a tight line without relying on hydrodynamic forces allows many possibilities outside the scope of conventional flow cytometry. In 2014, the next generation of acoustic cytometers is being released, featuring sample flow rates of up to 1 ml/min, acquisition speeds of up to 35,000 events/sec and up to 4 lasers and 16 optical parameters for detection. In this tutorial, we will demonstrate a unique No wash/No Lyse assay utilizing acoustic focusing and SSC from 2 different lasers to gate out unwanted RBCs. We will also discuss examples of using acoustic focusing to enable the detection of rare events by reducing centrifugation steps and retaining more cells. In addition we will examine cell cycle data acquired with flow rates from 12.5 ul/min to 1 ml/min and show that CVs do not vary with high flow rates when acoustic focusing is utilized. Join us to learn more on how acoustic focusing is enabling new applications in flow cytometry.
Adherent Imaging Cytometry Complementing Flow
Cytometric Measurements to Address the Diverse
Needs of a Research Environment

Romina Wiedmann
Cenibra GmbH, Germany

As more cell based assays are performed than ever before with samples that
are highly sensitive the need has never been greater for non destructive
methodologies for analyzing and quality controlling the growth and
characteristics of cell growth prior to downstream processing. Flow cytometry
has offered many technological developments and advancements in the field for
highly sophisticated and complex analyses. However, all flow cytometric
methods including imaging cytometric flow methods have specific limitations in
key areas due to the nature of flow cytometry requiring the samples in flow.
There is evidence for example that trypsination of cells can lead to a loss of cell
surface receptors and that non-disruptive morphological measurements may be
used as sensitive markers for profiling cellular behavior. The emergence of 3-D
models has also presented some specific challenges for traditional Flow
Cytometric methods. We explore adherent imaging cytometric here using the
Celigo S Imaging Cytometer, which allows many of these analyses to be
performed label-free and non-disruptively preserving the biological state, and
show the technology of adherent in situ imaging cytometry to be a suitable
complement to Flow Cytometric methods, with specific reference to Stem Cell
iPS generation and 3-D spheroid models.
24th Annual Conference of the German Society for Cytometry

October 15th-17th 2014
Center for Regenerative Therapies, Dresden

SESSION 7

Single Cell Analysis

Thursday - 16/Oct/2014
01:50 - 03:20 pm
Chairs:

**Thomas Kroneis**, Graz, Austria
**Vladimir Todorov**, Dresden, Germany

In many aspects, cytometry is best performed by means of flow cytometric analysis. However, in some instances spatial resolution as well as improved analysis at the level of single cells is highly requested.

In this session we will get insight into spatial resolution analysis at the single cell level based on a combination of protein analysis (*in situ* proximity ligation assay) and mRNA expression mapping in tissues. The ability to track pathways or the activation status of cells within its environment represents a major improvement in high-lighting site-dependent gradients within a sample and is thought to be a significant step towards deciphering complex biological interactions.

When heterogeneity at the single cell level was reported it became evident that scientific statements could only be given based on the statistical analysis of a large number of single cells. Addressing the significance of this the session will also include an introduction to single-cell qPCR and respective data analysis tools as well as the implementation of high throughput protein data measurement for the purpose of profiling.
Taking Expression Profiling to New Dimensions

Mikael Kubista
Institute of Biotechnology, Czech Academy of Sciences, Prague, Czech Republic and TATAA Biocenter, Göteborg, Sweden

qPCR is developing into the most powerful platform to profile samples, extracting the biologically relevant information. Major breakthrough in recent years is the development of robust pre-amplification methods, which allow the profiling of large number of markers starting with minute sample amounts, including single cells. In my talk I will describe high throughput single cell profiling of astrocytes from mouse brains performed over time after induced trauma. The profiling reveals how the brain responds to the injury, activating the astrocytes to attempt repair. Expression of many genes, measured in large number of cells, over time is a so called multiway study, from which most valuable information about the molecular processes involved and expression pathways active can be extracted with powerful multivariate tools such as dynamic PCA available in the GenEx software.
Cancer develops as a consequence of genetic mutations, which results in deregulation of gene expression and/or proteins with aberrant functions. Communication with other cells in the microenvironment will provide input signals that are interpreted by the malignant cells, and responded to, based on their altered genetic programs. Hence, the consequence of a mutation has to be viewed in the environmental context of each individual cell. The activity status of a protein or signaling pathway can be visualized with in situ Proximity Ligation Assays (in situ PLA) using a pair of antibodies equipped with DNA oligonucleotides (proximity probes) to target interacting proteins. Proximal binding of such probes template the creation of a circular DNA molecule, which is a surrogate marker for the interaction. We recently developed a multiplexed version of in situ PLA by introducing unique tags as identifiers in each different proximity probe. The combinatorial events generating an in situ PLA signal will harbor a set of identifier tags that will be unique for each protein interaction. By combining in situ PLA with padlock probes, analysis of signaling activity can be achieved together with genotyping expressed mRNA in fixed tissue sections, retaining the architectural information while providing single-molecule resolution. Detailed molecular characterization of tumors will enable personalized medicine, and may also be used in the development of novel treatments.
Heterogeneity of Brain Metastases Derived from Melanoma

Beate Rinner¹, Katharina Meditz¹, Marie-Therese Frisch¹, Sabrina Riedl², Helmut Schaider³, Bernadette Liegl-Atzwanger⁴, Karl Lohner², Dagmar Zweytick²

¹Center for Medical Research, Medical University of Graz; ²Institute of Molecular Biosciences, Biophysics Division, University of Graz; ³Dermatology Research Centre, The University of Queensland; ⁴Institute of Pathology, Medical University of Graz, Austria

Brain metastases, are one of the most common and difficult-to-treat complications of melanoma. Metastases are hard to treat, due to the fact, that brain might be a very natural environment for melanoma tumor to grow in, the blood-brain-barrier prevents many medications, and furthermore brain metastases tend to be very aggressive. Therefore new effective treatment approaches are urgently needed. We established and characterized a cell line derived from a melanoma brain metastasis. The cell line comprised morphologically heterogeneous cells. To separate the individual cell types, single cell sorting by FACS Aria were done and two clones developed. Each clone shows a unique phenotype and differs with regard to proliferation, migration, tumorigenicity and phosphatidylserine exposure. Recently we were able to demonstrate that the negatively charged lipid phosphatidylserine is specifically exposed by cancer cells and metastases and thus represents a uniform and novel target for cancer therapy.

Acknowledgment: Austrian Science Foundation FWF (grant no: P24608)
Picoliter Bioreactors: Learning from Single Cells about Large-Scale Bioprocesses

Alexander Grünberger, Christopher Probst, Wolfgang Wiechert, Dietrich Kohlheyer
Forschungszentrum Jülich GmbH, Germany

Our understanding of large-scale bioprocesses is still dominated by an average cell approach in which the reactor is well stirred and all cells behave equally. However, as confirmed in recent years, isogenic bacterial populations can be physiologically heterogeneous. Obviously, there is a strong demand to unravel microbial population heterogeneity, understand its origin and gain knowledge on its impact on large-scale biotechnological production. Therefore, new analytical techniques addressing single-cell behavior are the key for further optimization [1].

Picoliter bioreactors (PLBRs) for bacterial cells like those developed in our institute offer precisely controlled external conditions and allow single-cell analysis at full spatio-temporal resolution [2]. We present the versatility of our microfluidic single-cell analysis platform, which has been continuously improved over the last three years [1,2]. Furthermore, we will demonstrate how tailored designs allow for the analysis of specific biological questions on single-cell level. On selected examples we will show how picoliter bioreactors can be used to get a deeper understanding of microbial bioprocesses and cellular metabolism. Focus will be given on single-cell growth and production of Corynebacterium glutamicum, an industrial workhorse for amino acid production [3,4,5]. Finally, ongoing research topics such as the investigation of rare cellular events will be discussed.

Our results prove that PLBRs are a powerful tool to understand bioprocesses. Integrated into the existing bioprocess development pipeline, PLBRs provide the opportunity to gain single-cell knowledge of biotechnological processes essential for upcoming process optimization and research. Nevertheless, PLBRs are still in an early phase of their development, making a critical discussion of advantages, disadvantages, existing challenges and necessary improvements indispensable.

SESSION 8
Analysis of Big Data in Cytometry

Thursday - 16/Oct/2014
03:50 - 05:20 pm
Chairs:

Frank Schmidt, Greifswald, Germany
Christin Koch, Leipzig, Germany

The application of flow cytometry and high-throughput image based analyses makes the collection of large datasets of an unprecedented complexity possible. These datasets cannot be managed and processed in a manual manner any more. Together with the progress in other fields like DNA sequencing, transcriptome, proteome and metabolome analysis, today’s challenge is the multilevel data analysis and visualization. This session will present recent developments of new biostatistical and bioinformatics tools as well as mathematical modeling examples facilitating big data analysis for cytometric applications for a better understanding of biological systems.
Analyzing High-Throughput, Microscopy-based Cellular Screens

Lars Kaderali
Institute for Medical Informatics and Biometry, Medical Faculty, Technische Universität Dresden, Germany

High-content, high-throughput RNA interference (RNAi) offers unprecedented possibilities to elucidate gene function and involvement in biological processes. Microscopy based screening allows phenotypic observations at the level of individual cells. It was recently shown that a cell's population context significantly influences results. However, standard analysis methods for cellular screens do not currently take individual cell data into account unless this is important for the phenotype of interest, i.e. when studying cell morphology.

We present a method that normalizes and statistically scores microscopy based RNAi screens, exploiting individual cell information of hundreds of cells per knockdown. Each cell's individual population context is employed in normalization. We present results on two infection screens for hepatitis C and dengue virus, both showing considerable effects on observed phenotypes due to population context. Using a cell-based analysis and normalization for population context, we achieve improved sensitivity and specificity not only on a individual protein level, but especially also on a pathway level. This leads to the identification of new host dependency factors of the hepatitis C and dengue viruses and higher reproducibility of results.
Visualization Techniques for Big Data

Jörg Bernhardt
Institute for Microbiology, Ernst-Moritz-Arndt University
Greifswald, Germany

Due to the progress in DNA sequencing, transcriptome, proteome and metabolome analysis the life science community is faced with massive amounts of (semi) quantitative and often multi level categorized data. Classic visualization techniques do not support an effective data display anymore. By this reason we and others recently developed new tools supporting an overall and integrated presentation of data such as sets, categories and clusters, quantitative amounts, expression changes and many more. My talk will demonstrate this for several data sets, many of them related to cytometric applications.
Bioinformatical Approaches for the Analysis of Complex Microbial Communities

Joachim Schumann¹, Christin Koch¹, Susanne Günther¹, Ingo Fetzer², Susann Müller¹
¹Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Microbiology, Leipzig, Germany; ²Stockholm Resilience Centre, Stockholm University, Stockholm, Sweden

Flow cytometry (FCM) is used to measure optical characteristics of individual bacterial cells of natural communities in high-throughput. The resulting big data sets are visualized in cytometric histograms. These histograms represent the structure of microbial communities, e.g. at certain time points or microenvironmental conditions. Although flow cytometry is an important method in biological studies there are only very few tools for analyzing the variation in microbial community structures. New bioinformatical tools were developed in close collaboration of biologists and bioinformaticians to interpret the meaning of the structural variations. Up to now the most common tools are: FlowFP, Dalmatian Plot, Cytometric Histogram Image Comparison (CHIC) and Cytometric Barcoding (CyBar). The latter three tools were developed at the Helmholtz Centre for Environmental Research, Research Group Flow Cytometry.

The tools CHIC and CyBar are the most promising ones to create fingerprints for natural communities and get information on functions of cells within this community. To make the tools more accessible to the flow community a package was written in the statistical computer language R and published on the bioconductor online platform. Bioconductor is the most common used open software for bioinformatics and provides tools for the analysis and comprehension of many biological issues like flow cytometry, high-throughput sequencing and many more. The CyBar package was published in April 2014 and was downloaded 1079 times since the release (http://bioconductor.org/packages/release/bioc/html/flowCyBar.html). The CHIC package will be submitted soon. Both packages provide all applications needed for data evaluation as well as a detailed documentation and a user-friendly manual.

To make these two tools more easy to use a graphical user interface (GUI) was written in the object-orientated programming language JAVA for each tool. This GUI allows a fast and intuitive evaluation of the data even for those without any knowledge about programming.
Human Memory T Cells From the Bone Marrow are Resting and Maintain Long-Lasting Memory

Anna Okhrimenko, Joachim R. Grün, Kerstin Westendorf, Zhuo Fang, Simon Reinke, Philipp von Roth, Georgi Wassilew, Anja A. Kühl, Robert Kudernatsch, Sonya Demski, Carmen Scheibenbogen, Koji Tokoyoda, Mairi A. McGrath, Martin Raftery, Günther Schönrich, Alessandro Serra, Hyun-Dong Chang, Andreas Radbruch, Jun Dong

1 Cell Biology, Deutsches Rheuma-Forschungszentrum (DRFZ) Berlin; 2 Bioinformatics, Deutsches Rheuma-Forschungszentrum (DRFZ) Berlin; 3 Signal Transduction, Deutsches Rheuma-Forschungszentrum (DRFZ) Berlin; 4 Core Unit Cell Harvesting, Berlin-Brandenburg Center for Regenerative Therapies, Charité University of Medicine Berlin; 5 Center for Musculoskeletal Surgery, Charité University of Medicine Berlin; 6 Infectiology & Rheumatology, Charité University of Medicine Berlin; 7 Clinical Tumor Immunology & Immune-monitoring, Institute for Medical Immunology, Charité University of Medicine Berlin; 8 Osteoimmunology, Deutsches Rheuma-Forschungszentrum (DRFZ) Berlin; 9 Institute of Virology, Charité University of Medicine Berlin, Germany

Immunological memory is a central feature of the vertebrate immune system, which allows efficient and directed protection against secondary infections. Memory T cells, memory B cells and plasma cells build up the core of immunological memory, facilitating rapid clearance of recurring pathogens. Protective immunity against certain childhood infections can be stably maintained lifelong. In conflict with this fact, it has been shown that the number of antigen-specific memory T helper cells in blood steadily declines over time, suggesting a limited duration of recirculating T cell memory. However, the maintenance of immunological T cell memory remains unclear and represents one of the major questions in immunology.

In this work human peripheral blood and bone marrow memory T cells were extensively analyzed using multicolour flow cytometry. Immunophenotyping revealed a distinct population of memory T cells in bone marrow of healthy individuals, which express the surface molecule CD69. Despite the expression of CD69, expression of no other putative activation markers was detected. Furthermore, DNA content analysis and expression levels of Ki67, an antigen which is expressed during all active phases of the cell cycle, demonstrated that CD69 expressing memory T cells in bone marrow do not proliferate and preferentially rest in G0 stage.

The repertoire analysis of bone marrow resident memory T cells revealed their specificity against persistent, childhood and re-occurring antigens. We could demonstrate that memory T cells reactive to systemic pathogens are enriched in bone marrow and persist there, even after they have abandoned the blood circulation. These findings strongly suggest that T cell memory against systemic pathogens is mostly if not exclusively maintained in bone marrow, and thus, substantiate the new concept of resting systemic T cell memory.
SESSION 9

Nanobiotechnology / Nanobiomedicine

Friday - 17/Oct/2014
09:00 - 10:30 am
Chairs:

Wolfgang Fritzsch, Jena, Germany
Ulrike Taylor, Mariensee, Germany

The interaction of nanomaterials with organisms represents a field of growing interest due to the increasing use of nanoparticles in applications ranging from surface coatings via cosmetics to diagnostics and therapy. The session deals with this interaction on the level of cells and tissues, and will among others address the formation of particles, the interaction of particles with cells and their therapeutic use e.g. for drug targeting.
Nano-Sized Drug Delivery Systems for Nucleic Acids

Miriam Breunig, Luise Tomasetti, Eva-Christina Wurster, Renate Liebl, Achim Goepferich
Department of Pharmaceutical Technology, University of Regensburg, Germany

Many innovative medicines based on biological macromolecules such as nucleic acids are excluded from therapy due to insufficient availability at the site of action. A popular approach to rescue these substances for therapeutic applications has been to harness the opportunities provided by nanomaterials. Each nano-sized drug delivery system has to overcome various barriers on the tissue and cell level such as the extracellular matrix or the cellular membrane, respectively, on the way to their target cells. One poorly understood factor that significantly influences the transport of all nanomaterials across biological barriers are the physico chemical nanoparticle properties. Hence, we developed a nanoparticle platform that allows for systematically varying the size as well as their surface properties in a combinatorial approach. This strategy relies on the layer-by-layer technique, which involves the stepwise coating of nanoparticles with various polycations and polyanions. In addition, these nanoparticles can also be decorated with hydrophilic molecules such as poly (ethylene glycol) (PEG) to avoid unspecific interactions with non-target cell and to improve their transport properties in the extracellular matrix.

The talk will focus on the development of these materials and will provide results that we obtained from cellular uptake studies. Concomitantly the distribution and transport of these nanoparticles in three-dimensional tumor spheroids will be illustrated. This progress in the field of delivery technology will substantially help us to make better use of nucleic acids for therapeutic applications.
Diagnostic Nanoparticle Targeting of the EGF-Receptor Using Single-Domain Antibodies

Kristof Zarschler¹, Kanlaya Prapainop², Eugene Mahon², Louise Rocks², Mattia Bramini², Phil M. Kelly², Holger Stephan¹, Kenneth A. Dawson²
¹Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Germany; ²Centre for BioNano Interactions (CBNI), University College Dublin, Ireland

For effective localization of functionalised nanoparticles at diseased tissues such as solid tumours or metastases through biorecognition, appropriate targeting vectors directed against selected tumour associated antigens are a key prerequisite. The diversity of such vector molecules ranges from proteins, including antibodies and fragments thereof, through aptamers and glycans to short peptides and small molecules. Here, we analyse the specific nanoparticle targeting capabilities of two previously suggested peptides (D4 and GE11) and a small camelid single-domain antibody (sdAb), representing potential recognition agents for the epidermal growth factor receptor (EGFR). We investigate specificity by way of receptor RNA silencing techniques and look at increasing complexity in vitro by introducing increasing concentrations of human or bovine serum. Peptides D4 and GE11 proved problematic to employ and conjugation resulted in non-receptor specific uptake into cells. Our results show that sdAb-functionalised particles can effectively target the EGFR, even in more complex bovine and human serum conditions where targeting specificity is largely conserved for increasing serum concentration. In human serum however, an inhibition of overall nanoparticle uptake is observed with increasing protein concentration. For highly affine targeting ligands such as sdAbs, targeting a receptor such as EGFR with low serum competitor abundance, receptor recognition function can still be partially realised in complex conditions. Here, we stress the value of evaluating the targeting efficiency of nanoparticle constructs in realistic biological milieu, prior to more extensive in vivo studies.
Introduction to a Multidisciplinary Approach for the Safe Implementation of Nanotechnology in the Environment

Lutz Mädler
Foundation Institute of Materials Science, Department of Production Engineering, University of Bremen, Germany

In the safety assessment of commercial nanoparticles (metal oxides) it is required that we understand how the physicochemical characteristics of the engineered nanomaterials relate to biological responses such as cellular uptake, biodistribution, bioavailability and the catalysis of hazardous biological responses at the nano/bio interface.

Using 24 representative metal oxides, we demonstrate that the toxicity of metal oxide nanoparticles closely correlates with their semiconducting property and band positions. Overlap of metal oxide conduction band energies (Ec) with the cellular redox potential (-4.12 to -4.84 eV) can lead to ROS generation and oxidative stress injury as well as the generation of pulmonary inflammation, which can be assessed by single- and multi-parametric toxicological assay as well as acute pulmonary inflammation. While the toxicity of CuO and ZnO is independent of their Ec levels, the adverse biological effects of these materials could be explained by their solubility. These results provide a novel platform for establishing MOx toxicity.

These results demonstrate that it is possible to predict the toxicity of a large series of MOx nanoparticles in the lung premised on semiconductor properties and an integrated in vitro/in vivo hazard ranking model premised on oxidative stress. This establishes a robust platform for modeling of MOx structure-activity relationships based on band gap energy levels and particle dissolution. We also demonstrate that the in silico hazard ranking and statistical tools can be used to establish a predictive toxicological paradigm, in which in vitro toxicological ranking can be used to predict the in vivo toxicological outcome. This predictive toxicological paradigm is also of considerable importance for regulatory decision-making about this important class of engineered nanomaterials.

These studies have been conducted in close collaboration with other partners from the Center of Environmental Implications of Nanotechnology (CEIN).
SESSION 10

Functional Heterogeneity in Microbial Processes

Friday - 17/Oct/2014
10:50 - 00:20 pm
Chairs:

Susann Müller, Leipzig, Germany
Christian Dusny, Dortmund, Germany

New methods for single cell analysis show improved possibilities not only for the in depth analysis of individual cells but also to investigate what is happening in microbial populations and communities. Today, there are several powerful technologies available to investigate and describe the dynamics of the cells in populations or communities. Completed by other techniques it extends the understanding of structure and function relationships by investigating phylogenetic, genomic, transcriptomic and proteomic levels at the scale of the single microorganism or that of sub-communities. The new information collected helps to better understand the functioning of the cells and their intra- or inter-relationships. The session will contribute to reveal principles of microbial ecosystems or to interpret even simpler systems like pure cultures as are used in microbial (white) biotechnology. Potential applications will also be addressed.
The single cell represents the basal self-sustaining unit of life. Each single cell constitutes an independent and complex system that shows remarkable individuality in a multitude of physiological traits. Consequently, a microbial population exhibits physiological diversity despite of clonality, which can be exclusively accessed by single cell analysis. This especially concerns the cellular repertory of adaptation mechanisms reacting to physicochemical changes in the extracellular environment. Such mechanisms include stochastic alterations of regulatory circuits as well as specific adaptations to external stimuli, which are highly individual and strongly depend on frequency and extent of fluctuations in the cells surrounding environment. Therefore, the control of the extracellular (micro-) environment is of fundamental importance for the unambiguous identification of the origins of intrapopulation heterogeneity of an actually clonal population. Only with this, the occurrence of cell-to-cell differences can be assigned to either environmental factors or intrinsic stochasticity. To date, only microfluidic single cell cultivation systems allow the necessary degree of control over the extracellular environment. In this talk, we present a systematic comparison of principally different cultivation technologies in order to quantify cellular responses to the environment at a single cell level. We compared contactless cell retention based on negative dielectrophoresis (nDEP), allowing constant environmental conditions by continuous cell perfusion, with flow-through contact-based hydrodynamic single-cell cultivation systems (MGC) and static solid agarose-based cultivation pads. To enable a quantitative comparison of these three systems on the basis of physiological and morphological parameters, universally applicable analytical approaches for the precise quantification of specific growth rates, cell morphology and division characteristics of single microorganisms were developed. Corynebacterium glutamicum ATCC13032 was harnessed as a model organism and cultivated under otherwise identical cultivation conditions with all three systems. Interestingly, all cultivated cells exhibited virtually identical growth capacity and showed exceptionally robust and high specific growth rates of 0.6-1 h for micropopulations of up to 8 cells, independent of the applied cultivation technology. The observed growth rates of single cells exceeded population growth rates in shake flasks by up to 120 %. These results lead to the conclusion that optimal nutrient supply was present with all employed systems and the rate of growth of a cell is tightly regulated and conserved. It could therefore be argued that the metabolic capacity of the cells is rate-limiting rather than environmental factors. Unlike growth rates, division rates, snapping division angle and cell length distributions showed considerable differences in the static environment of agarose pads, whereas cells cultivated with nDEP and MGC had similar length and division angle distributions. To the best of our knowledge, this is the first investigation of physiological responses to steady and static extracellular environments at the single cell level. Moreover, this study underlines the importance of exploring cellular physiology at a single cell level, eliminating bias by uncontrollable fluctuations in the extracellular environment and population activity.
Plasmid Copy Number Variation in *Pseudomonas putida* Analyzed by Cell Sorting and Digital Droplet PCR

Michael Jahn¹, Carsten Vorpahl¹, Dominique Türkowsky¹, Martin Lindmeyer², Bruno Bühler², Hauke Harms¹, Susann Müller¹

¹Helmholtz-Centre for Environmental Research - UFZ, Leipzig; ²Laboratory of Chemical Biotechnology, TU Dortmund University, Germany

Whole microbial cells are cheap, efficient and self-reproducing catalysts for the enzymatic conversion of chemicals or production of proteins. For expression of the gene of interest plasmids are widely used in academia and industry. However, the overall yield and efficiency of production processes is easily disturbed by fluctuations in plasmid copy number (PCN), leading to population heterogeneity. Here, we investigated the effect of PCN on heterologous expression of a model enzyme in *Pseudomonas putida*. Conventionally, plasmid retention is estimated by plate counting on selective media or roughly determined by gel electrophoresis. In contrast to that, we used an EGFP reporter fused to the target protein, allowing real time fluorescence screening of individual cells by flow cytometry. Furthermore, we developed a work flow for direct PCN analysis using cell sorting and cutting edge Droplet Digital PCR (ddPCR). The work flow using a duplex TaqMan® ddPCR setup was tested and optimized for as little as 1,000 sorted cells, and gave insights into population heterogeneity on the DNA level not seen before.

We found, that a large proportion of cells (30-60 %) was not able to produce the desired protein even under high induction regimes, dividing the population into high- and low-fluorescent cells. The ddPCR analysis revealed that plasmid distribution was remarkably unequal, directly correlating the strength of EGFP expression to low and high PCN. We further exploited this technique to probe the influence of antibiotics on PCN, as well as the combination of different expression plasmids and microbial strains to select an optimal production system.
Flow Cytometry for Energy Balances of Phytoplankton Organisms

Susanne Dunker, Torsten Jakob, Christian Wilhelm
University of Leipzig, Germany

Phytoplankton organisms (cyanobacteria and algae) are microscopic small organisms having an enormous impact on the water quality and ecology in waters. Their ability to convert light energy to biomass via photosynthesis determines the extent of primary production. The efficiency of this conversion highly varies within the taxonomic groups (e.g. cyanobacteria, green algae, dinophytes, cryptophytes and diatoms). This means that the same quantity of light not necessarily leads to the same amount of biomass for different taxa. In contrast to green algae, cyanobacteria can use extremely low light intensities for biomass production but due to their toxicity they are not an appropriate food source for primary consumers and decrease water quality. Until now a resolution of energy use efficiency in a complex community for different taxa is not possible, but quite important for water-quality-management. The idea behind the project was to use cellular characteristics to resolve the energy use efficiency for different species in a mixed culture. On a basic level we cultured the cyanobacterium (*Microcystis aeruginosa*) with one of two green algae (*Scenedesmus obliquus* or *Oocystis marsonii*). By the means of flow cytometry (FC) both species in a mixed culture could be distinguished due to their different pigmentation. FC delivered the following cell parameters: cell volume, dry weight and chl a -content per cell. These data allowed the quantification of absorbed light energy in relation to created biomass separately for the species in the mixed culture. It could be shown that energy use efficiency (numbers of photons needed to assimilate one molecule of carbon) of the green algae *Oocystis marsonii* in a mixture with *Microcystis aeruginosa* was strongly reduced in comparison to uni-algal control culture, while energy use efficiency of *Scenedesmus obliquus* was unaffected in growth by the mixture with the cyanobacterium. In consequence it could be shown how energy is distributed and converted into biomass in a phytoplankton community. The estimation of these taxonomical resolved energy balances was only possible by the means of flow cytometry. In future this single-cell-approach could be a valuable tool for water-quality-management.
Enhanced Viability of Microalgal Populations by Photoperiodic Cycles

Felix Krujatz, Thomas Bley, Jost Weber
TU Dresden, Institute of Food Technology and Bioprocess Engineering, Germany

Microalgae represent a promising raw material for various industries due to their wide range of valuable ingredients. By photosynthetic processes carbon dioxide is fixed under the influence of light and converted into products like proteins, pigments, biopolymers, lipids or drugs. Despite the great potential of algae research only 150 of the estimated 400,000 algae strains are used for industrial applications.

Basically, cultivation of microalgae is performed in open or closed photobioreactor systems, varying in size and geometry. Light energy for photosynthetic processes is commonly provided by external or internal illumination devices. Self-shading and inhomogeneous illumination of photobioreactors are responsible for light gradients within microalgae suspensions which consequently causes the formation of population heterogeneity. It has been proven that population heterogeneity of microbial cultures can significantly influence the productivity of biotechnological processes. An important cell-specific parameter for the overall productivity of microbial cultures is given by the viability which can be defined as the differentiation of metabolic active and inactive cells. *Chlamydomonas reinhardtii* 11.32b and *Chlorella sorokiniana* UTEX1230 are widely used and robust model organisms for photosynthesis and algae research due to their high growth rates and temperature resistance. In this study, a flow cytometry viability staining procedure was established using bis-(1,3-dibarbituric acid)-trimethine oxonol (DiBAC4(3)), a slow response membrane potential sensitive probe. Above mentioned microalgal strains were cultivated in shake flasks under varying temperature (26°C, 30°C and 37°C) and photoperiodic light cycles (150 µmol m-2 s-1, ligt/dark: 24/0 or 14/10). We found that for all culture conditions *C. sorokiniana* UTEX1230 obtained higher cell densities compared to *C. reinhardtii* 11.32b even at 30°C and 37°C. In comparison to full light conditions (light/dark cycles of 24/0) photoperiodic cycles of 14/10 hours resulted in lower cell densities of *C. reinhardtii* 11.32b and *C. sorokiniana* UTEX1230 at all considered cultivation temperatures.

After a period of stable population viability (ca. 50 hours) the amount of DiBAC4(3) positive *C. reinhardtii* 11.32b and *C. sorokiniana* UTEX1230 cells increased at 30°C and 37°C. For both strains it could be observed, that the viability of microalgal populations remained at constant high values by adjusting photoperiodic cycles of 14/10 hours.

Thus, the amount of metabolic active cells can directly be influenced by photoperiodic cycles.
Staphylococcus aureus Infection Induces Human T Cell Apoptosis and Increased Mortality in Humanized Mice

Anja Kathrin Wege¹, Janin Knop¹, Frank Hanses², Nancie Archin³, Joachim Gläsner⁴, Andre Gessner⁴
¹University Medical Hospital Regensburg, Department of Gynecology and Obstetrics, Germany; ²Medical Hospital Regensburg, Department of Internal Medicine, Germany; ³University of North Carolina at Chapel Hill, Department of Medicine, USA; ⁴University of Regensburg, Institute for Medical Microbiology and Hygiene, Germany

Background: S. aureus is a common pathogen causing infections in humans worldwide with an increasing prevalence of multidrug resistance. Unfortunately, mouse and human immune systems exhibit some substantial differences and the immune responses against pathogens and thereby the outcome of infectious diseases differs dramatically between species. As many as 150 clinical studies failed in clinical settings although they showed promising results in mice studies. In addition, the rate of methicillin-resistant S. aureus (MRSA) strains is increasing and therefore, new treatment strategies and an appropriate animal model to test these approaches under human-like conditions are urgently needed.

Methods: In this study we established an S. aureus infection model in mice engrafted with a human immune system and investigated the role of apoptosis induction for pathogenesis.

Results: S. aureus infection was aggravated in these humanized mice (HM) compared to wild type or non-engrafted mice. The HM displayed significantly reduced survival, increased weight loss and accelerated bacterial burden. S. aureus infection in HM elucidated the negative impact of a human immune response on disease progression which was characterized by increased human cytokine production, T cell apoptosis and FAS receptor expression.

Conclusion: Our findings clearly demonstrate the different effects obtained in wild type and humanized mice and discuss the possible benefit of including humanized mice in future studies involving S. aureus as a prior step to human clinical trials.
SESSION 11

Klaus-Goerttler-Session - Immunology

Friday - 17/Oct/2014
01:00 - 02:00 pm
Chair:

Petra Bacher, Berlin, Germany
Gergely Toldi, Budapest, Hungary

The first two presentations of this session will focus on novel applications of flow cytometry employing disease-specific signatures and biomarkers with a potential for monitoring immune responses. The role of extracellular vesicles has gained increasing attention in the field of autoimmune disorders over the recent years. These particles may be used as specific targets for flow cytometry measurements. Type I Interferon responses are important aspects not only during viral infections, but also in autoimmune disease. Monocytes are sensitive biosensors to monitor type I interferon responses, and with the help of recently established novel flow cytometry markers, they may also be applied in a clinical setting.
Detection of Extracellular Vesicles in Inflammation

Edit Buzás
Department of Genetics, Cell- and Immunobiology,
Semmelweis University, Budapest, Hungary

Earlier work of her group has demonstrated that the presence of protein aggregates (immune complexes, avidin-biotin complexes or antibody self-aggregates) may result in extracellular vesicle-mimicking signals during flow cytometry. A simple universal method was introduced to differentiate extracellular vesicles from non-vesicular events: differential detergent lysis. Low concentration (0.05-0.1%) Triton X100 lyses membrane bound vesicles whereas protein aggregates are more resistant to detergent lysis.

By applying differential detergent lysis to exclude non-vesicular events during enumeration of microvesicles/microparticles by flow cytometry, the group identified disease-specific extracellular vesicle signature in the synovial fluid of patients with rheumatoid arthritis. Rheumatoid arthritis patient-derived synovial fluid samples were found to contain significantly elevated number of CD3+ and CD8+ microvesicles.

The group has demonstrated that *in vitro* vesiculation related to blood sample handling and storage was prevented in ACD anticoagulant tubes. Importantly, microvesicle levels elevated *in vivo* remained detectable in ACD tubes. Therefore ACD tubes were suggested for general use for the assessment of blood plasma microvesicles by flow cytometry.

Current interest of her group focuses on spectroratiometric assessment of membrane lipid order in different types of extracellular vesicles as well as on identification of novel markers for identification of extracellular vesicle subtypes.
Monocytes as Biosensors of Type I Interferon Responses in Autoimmunity and Viral Infection

Andreas Grützkau
German Rheumatism Research Center Berlin (DRFZ), Immune Monitoring, Berlin, Germany

Type I interferon responses are typically induced during viral infections, but are also observed as a hallmark in the pathophysiology of autoimmune diseases, such as SLE or scleroderma. So, type I interferon-associated molecules or signatures are of major interest as biomarkers that would help to improve diagnosis and therapy stratification of these chronic diseases. Here we show how cell-specifically generated whole genome transcriptome analyses of peripheral blood monocytes can be used to differentiate between physiological and pathophysiological IFN responses. Furthermore, we will give an example how this knowledge is translated to establish biomarkers, such as SIGLEC-1 (CD169), which can be used in a clinical setting by flow cytometry to monitor disease activity of lupus patients in daily routine.

It was striking that all of the transcripts that were regulated in response to viral exposure were also found to be differentially regulated in SLE, albeit with markedly lower fold-change values. In addition to this common IFN signature, a pathogenic IFN-a-associated gene signature was detected in the monocytes from lupus patients. Type I IFN signatures identified were successfully applied for the monitoring of interferon responses in PBMCs of an independent cohort of SLE patients and virus-infected individuals. Moreover, these cell-type specific gene signatures allowed a correct classification of PBMCs independent from their heterogenic cellular composition. SIGLEC-1 (CD169) was one of the most prominent genes found to be differentially regulated in SLE patients. We could show that its expression correlates with disease activity and outperformed gold standards in lupus diagnostic.

In conclusion, our data showed for the first time that monocytes are sensitive biosensors to monitor type I interferon responses in autoimmunity and viral infection and how these transcriptional responses are dysregulated in SLE. Based on the IFN-associated gene signatures identified SIGLEC-1 emerged as a promising surrogate marker for type I interferon responses, which will help to monitor disease activity, prediction of flares and to adjust anti-IFN-alpha directed therapies in terms of a personalized medicine approach.
The inositol 1,4,5-trisphosphate receptor (IP$_3$R) is an important mediator for the Ca$^{2+}$ release from the endoplasmic reticulum (ER) and its function is negatively regulated by the NO/cGMP/cGMP kinase Iβ (cGKβ) signaling pathway. cGKβ phosphorylates the IP$_3$R-associated cGMP kinase substrate (IRAG) which in turn inhibits the IP$_3$R1 mediated calcium release from intracellular stores. These pathways are also involved in the activation cascade of T lymphocytes: Several effects of cGMP and cGKI in T cells have been reported including modulation of the cytokine production in Th2 cells [1], inhibition of IL-2 release and inhibition of T cell proliferation [2] and the induction and stability of Th17 cells [3].

To investigate the influence of IRAG in the context of T cell activation, we characterized IRAG$^{-/-}$ mice in more detail. These mice developed a strong splenomegaly that is not related to platelet development as shown with a platelet specific knock out mouse. The expression of IRAG and cGKβ in T cells was confirmed by Western Blot analysis of T cells isolated from wild type (WT) mouse spleens. FACS analysis of primary IRAG$^{-/-}$ splenocytes showed a decreased percentage of CD4$^+$ lymphocytes but apart from that a normal distribution of lymphocyte populations. Further determination of CD4$^+$ subsets showed a distinct T helper phenotype compared to WT mice. FACS analysis of CD4 T cell subpopulation revealed a 2-fold increased number of follicular T helper cells (Tfh) whereas the numbers of Th2 and Th17 cells were considerably lower than those found in age-matched WT mice. There were no significant differences in the Th1 and Treg numbers of IRAG$^{-/-}$ and WT animals. In conclusion, these observations imply a role of IRAG during T cell differentiation and maturation that should be investigated in more detail in additional experiments.

1 B. Gomes, M. Savignac, M. D. Cabral J Biol Chem 2006
Chairs:

Hyun-Dong Chang, Berlin, Germany
Axel Roers, Dresden, Germany

Immune responses are a complex spatial and temporal interplay on many different cell types. The identification of the cellular correlate of protective immunity and/or immunopathology often requires the analysis of rare cell types, namely those which specifically respond to a particular antigen. In this session innovative technologies will be discussed which allow us not only to monitor immune responses on the single cell level, but also to understand mechanisms of protection and pathology and to develop cellular therapies for infectious diseases, autoimmunity, and cancer.
Cytometric Analysis of Antigen-Specific T helper Cell Responses as Diagnostic Sensors in Infection and Allergy

Alexander Scheffold
Department of Rheumatology and Clinical Immunology
Charité - University Hospital Berlin & German Rheumatism Research Center, Berlin, Germany

Antigen-specific T helper (Th) cells orchestrate adaptive immunity and are therefore critically involved in immune protection as well as immunopathology. The quantitative and qualitative characterization of antigen-specific T helper cell responses has great potential for diagnosis as well as for the development of more specific immune intervention strategies against infections, tumors, autoimmunity, allergy and other chronic inflammatory diseases. However, the analysis of antigen-specific Th cells especially in humans is limited by their low frequency and the few defined antigenic epitopes. This is particularly true for complex pathogens, such as bacteria or fungi, which express hundreds or thousands of potential antigenic proteins.

We have developed a sensitive assay for rare antigen-specific T helper cells based on magnetic enrichment of in vitro antigen-activated T cells. Using CD154 and CD137 as specific markers, which are expressed by in vitro antigen-activated conventional and regulatory T cells, respectively, both subsets can simultaneously be identified. Due to the high sensitivity of the assay even the naive T cell repertoire can be analyzed. The parallel assessment of the total antigen-specific repertoire, i.e. conventional naive and memory T cells together with Treg, allows precise determination of the specific immune status against basically any antigen of interest.

I will give an overview how we have used this versatile and sensitive technology to characterize antigen-specific T cells in various human immune pathologies, like infectious diseases, allergies, autoimmunity or inflammatory bowel disease. Our data suggest that antigen-specific T cells can be used as precise patient-specific sensors of the host-pathogen interaction status, which significantly contributes to our basic understanding of complex immune-mediated processes and will improve diagnostic or therapeutic applications of immune pathologies.
In the meantime it is well accepted that CD8\(^+\) T cells play a pivotal role in providing protection against infection with intracellular pathogens and some tumors. Based on these observations, the development of adoptive cell therapies (ACT) using highly defined T cell products is currently gaining substantial clinical interest. Recent data indicate that most effective ACT requires in vivo expansion as well as long-term maintenance of transferred cells. Different subsets of antigen-specific effector and memory CD8\(^+\) T cell have been described, which seem to differ substantially in their capacity to reconstitute protective immunity upon ACT. Although it becomes evident that clinical pre-enrichment for defined subsets might help to make engraftment and maintenance of T cells upon ACT more predictable, it is still controversially discussed which subset to choose. Furthermore, since T cell subsets are usually defined by the expression of multiple cell surface molecules, novel approaches facilitating clinical multi-parameter cell processing and purification are needed.

Over the past years we have developed single-cell adoptive transfer technologies combined with reversible cell labeling (so-called Streptamer technology) and polychromatic multiparameter flow cytometry of rare event populations. This novel type of single cell fate mapping has allowed us to demonstrate unequivocally that some individual precursor cells within the naive and memory T cell pool can bear the full plasticity to develop into a plethora different T cell subsets (stem cell like plasticity). Furthermore, we can demonstrate that to a defined memory T cell subset all characteristics of adult tissue stem cells can be attributed. These novel observations have important implications for ACT since they demonstrate how it is possible to reconstitute effector and memory populations from very low numbers of adoptively transferred T cells. We are currently exploring Streptamer-based cell purification platforms to enable clinical purification of most effective T cell populations for ACT.
B7 Costimulation and Intracellular Indoleamine 2,3-Dioxygenase (IDO) Expression in Umbilical Cord Blood and Adult Peripheral Blood

Gergely Toldi, Enikő Grozdics, László Berta, Tivadar Tulassay
Semmelweis University, Budapest, Hungary

Objectives: Alterations in the expression of B7 costimulatory molecules and their receptors as well as differences in the tryptophan catabolic pathway may influence immunological reactivity of umbilical cord blood (UCB) compared to adult peripheral blood (APB) T lymphocytes.

Methods: We determined the frequency of activated (CD11b+) monocytes expressing B7-1, B7-2, B7-H1, and B7-H2, and that of T cells and CD4+ T helper cells expressing CD28, CTLA-4, PD-1, and ICOS in UCB and APB samples using flow cytometry (BD FACS Aria). We also examined the intracellular expression of indoleamine 2,3-dioxygenase (IDO) applying flow cytometry and plasma levels of tryptophan (TRP), kynurenine (KYN) and kynurenic acid (KYNA) using high-performance liquid chromatography.

Results: The level of CTLA-4 expression on CD4 cells was higher in UCB compared to APB, indicating that the possibility of CD28-mediated costimulation may be decreased. The level of the corresponding costimulator molecule, B7-2 was also elevated. Therefore, this inhibitory relation may function to a higher extent in UCB than in APB. The plasma KYN to TRP (K/T) ratio was two-fold higher in UCB compared to APB. However, the capacity of UCB monocytes compared to APB monocytes was lower to produce IDO, and reverse signalling via B7-2 in UCB monocytes was found to be immature, which suggests that the observed increase in K/T ratio may be due to placental rather than fetal overexpression of IDO in competent cells.

Conclusion: These factors may all contribute to the previously observed reduced reactivity of UCB T lymphocytes compared to APB T cells.
SESSION 13

Meet the Expert Lecture

Friday - 17/Oct/2014
03:10 - 04:00 pm
Chairs:

Leoni A. Kunz-Schughart, Dresden, Germany
Andreas Deutsch, Dresden, Germany

Dr. Hynynen is a graduate of the Univ. of Kuopio, Finland, where he obtained his Bachelor & MSc degrees in Physics. He earned his Doctorate (PhD) in Biomedical Physics and Biomedical Engineering in 1982 from the Univ. of Aberdeen, UK.

In 1992, Dr. Hynynen was the first to show the feasibility of MRI guided focused ultrasound surgery. He is currently the Director of Physical Sciences Platform at Sunnybrook Research Institute and a Professor in the Dept. of Medical Biophysics at Univ. of Toronto, Canada. He holds a Canada Research Chair in Imaging Systems and Image-Guided Therapy and leads the Centre for Research in Image-Guided Therapeutics.

Dr. Hynynen's research focuses on studying the effects of ultrasound beams on tissue and their utilization in therapy. He is investigating the use of focused ultrasound for noninvasive surgery, vascular surgery, targeted drug delivery and gene therapy. He has developed theoretical models to help understand treatment parameters and to allow optimization of treatment devices. He has conducted in vivo experiments to investigate the various biological endpoints that can be induced by controlling the sonication parameters. His research on ultrasound transducers has been used to design and develop new phased array applicators and better ways to deliver therapy. He is also looking at the use of magnetic resonance imaging to detect and quantify noninvasive ultrasound exposure in vivo and has developed several systems that are being tested clinically.
The Feasibility of Using Ultrasound for Transcranial Imaging and Targeted Drug Delivery in the Brain

Kullervo Hynynen$^{1,2}$  
$^1$Department of Medical Biophysics, University of Toronto, Ontario, Canada; $^2$Physical Sciences Platform, Sunnybrook Research Institute, Toronto, Ontario, Canada

Recent development in ultrasound methods have made noninvasive focusing of ultrasound into deep targets in the brain possible. This method is now used under Magnetic Resonance Imaging for the treatment of essential tremor, chronic pain and Parkinson's disease. However, the method when combined with standard diagnostic ultrasound imaging contrast agents containing micron sized gas bubbles, has shown great promise for focal opening of the Blood-Brain barrier (BBB) for targeted drug and cell delivery. The bubbles that are injected in the blood stream act as energy concentrators and insert significant physical stretching on the capillary walls when the bubbles expand and contract with the pressure wave. Therefore very low time average powers (in the order of a few mWs) are deposited in the brain thus having minimal impact on brain tissue. The BBB heals itself in approximately in 6 h but is dependent on the amplitude of the acoustic pressure. A wide variety of therapeutic agents have been delivered in the brain in animals ranging from chemotherapy agents to antibodies to stem cells. The treatments have been tested in disease models and found effective against for example brain tumours and Alzheimer's Disease. It is expected that the clinical testing of this method may start in the near future.

The skull aberration correction methods and the multi-element ultrasound phased arrays that have made through skull sonications possible can be used also for brain imaging with high resolution. The ultrasound excited microbubbles also emit ultrasound that can be detected outside of the skull with sensitive receivers. By using a large number of receivers distributed all around the skull, the bubbles can be localized. The localization of sound sources has been used in acoustics and is called Passive Acoustic Mapping (PAM). By incorporating skull-specific aberration corrections into a conventional PAM algorithm allows microbubble echoes to be imaged through the skull. By utilizing super-localization methods originally developed for optical microscopy, high resolution images of the bubbles can be created though the skull. The phantom images obtained through a human skull show promise and indicate that the method may eventually be used for the mapping of the complete brain vasculature.
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P1 - P40

Poster Session I:
Thursday-16/Oct/2014, 12:20 - 01:05 pm
Odd numbers

Poster Session II:
Thursday-16/Oct/2014, 01:05 - 01:50 pm
Even numbers
Prospective Isolation and Characterization of Human Bone Marrow-derived Mesenchymal Stem/Progenitor Cells and Identification of Distinct Subsets

Sabrina Grimm, Gloria Altea Moya, Hans-Jörg Bühring
Laboratory for Stem Cell Research, University Clinic Tübingen, Germany

Bone marrow-derived Mesenchymal Stromal/Stem cells (MSC) are non-hematopoietic cells, which are able to differentiate into osteoblasts, adipocytes and chondrocytes. In addition, they are known to participate in the niche formation for hematopoietic stem cells (HSC) and to display immunomodulatory properties. Conventionally, these cells are functionally isolated from tissue based on their capacity to adhere to the surface of culture flasks. This isolation procedure is hampered by the unpredictable influence of secreted molecules and interactions with co-cultured hematopoietic and other unrelated cells as well as by the arbitrarily selected removal time of non-adherent cells prior to expansion of MSC. Finally, functionally isolated cells do not provide biological information about the starting population. To circumvent these limitations, several strategies have been developed to facilitate the prospective isolation of MSC based on the selective expression or absence of surface markers. Here we summarize the most frequently used markers, introduce new targets for antibody-based isolation procedures and identify subsets of primary bone marrow-derived MSC.
P2 Communication Between Hematopoietic Stem and Progenitor Cells

Doreen Reichert¹, Julia Scheinpflug¹, Martin Bornhäuser²,³, Denis Corbeil¹²

¹Tissue Engineering Laboratories (BIOTEC); ²Center for Regenerative Therapies Dresden (CRTD); ³Medical Clinic and Polyclinic I, University Hospital Carl Gustav Carus, Dresden, Germany

Cell-to-cell communication is a crucial prerequisite for the development and maintenance of multicellular organisms. Thin plasma membrane-derived tubes (referred to as tunneling nanotubes or nanotubular highways (NTHs)) have been described in different cell types. They can bridge cells in distance up to hundred micrometers and might have an important role in communication, especially for the selectivity and velocity of specific signaling pathways. The transport of numerous cargo molecules along NTHs has been reported in various biological processes including development, immune defense, cancer progression and pathogen spreading. Electric signal could also be transferred (or sensed) between connected cells.

Here, we observed that hematopoietic cells such as KG1a cells and CD34⁺ granulocyte-colony stimulating factor (G-CSF)-mobilized hematopoietic stem and progenitor cells (HSPCs) display NTHs. As monitored by scanning electron microscopy, NTH ending points appear mainly to be closed in the case of KG1a cells whereas they seem to be open (i.e. fused) in the case of CD34⁺ HSPCs. Biochemically, NTHs are based on actin as determined by phalloidin labeling and their sensitivity to the actin depolymerization agent Latrunculin B. They are insensitive to the microtubule depolymerization agent Nocodazole suggesting that they are no relicts of incomplete cytokinesis. Mechanically, NTHs are mainly formed de novo by adjacent cells coming into contact and then moving apart rather than by a directed outgrowth of a filopodia-like protrusion toward a neighboring cell. Do hematopoietic cells use NTHs to communicate with each other? Indeed, we revealed during time-lapse microscopy that the stem cell marker CD133 is “clustered” along the NTHs and transferred unidirectional from one cell to another. In contrast, other membrane proteins (e.g. CD34, CD44, CD53 and CD63) where evenly distributed along the NTH.

Collectively, cell migration appears to be a requisite in biogenesis of NTHs, and their formation in vivo might be related to the migration of HSPCs within bone marrow niches. Moreover, the directed transfer of cargo molecules, notably CD133, via NTHs may influence the cell fate in a similar way as the release of CD133-containing membrane vesicles by HSPCs, concomitant with their differentiation.
Regeneration of Renin Cells during Nephrogenesis and in Adult Life

Linda Hickmann, Jan Sradnick, Charlotte Starke, Peter Lachmann, Anika Lüdemann, Bernd Hohenstein, Christian Hugo, Vladimir Todorov
University Hospital Carl Gustav Carus Dresden, Germany

Renin is the rate-limiting factor of the renin-angiotensin-system, which plays a central role in the regulation of blood pressure, salt/water homeostasis and kidney development. Whereas in the adult the enzyme is produced only in the juxtaglomerular cells of the kidney, during development it is expressed in many other cells that differentiate into vascular smooth muscle cells, mesangial cells and collecting duct cells. However, it is still unknown, if during development there is a population of progenitor cells which first become renin positive and then further proliferate or if there is a persisting pool of precursors differentiating into renin-producing cells. To study this issue we used a transgenic mouse model allowing the precise tracking of the neogenesis of renin cells.

Mice expressing cre recombinase under the control of the endogenous renin promoter (mRenCre) were crossed with tdTomato-eGFP mice. The double transgenic mRenCre-tdTomato-eGFP mice switch the tdTomato into eGFP expression in renin-producing cells. Newly differentiated renin cells are positive for both tdTomato and eGFP, whereas earlier differentiated cells are only eGFP positive. The kidneys of different developmental stages of these mice were analyzed using FACS and immunohistochemistry.

The first eGFP positive cells were observed on embryonic day 14. All of them were positive for tdTomato and renin, too. On embryonic day 16 the first eGFP positive/tdTomato negative and eGFP positive/renin negative cells were detected. During nephrogenesis the total number of eGFP positive cells increased, whereas the amount of tdTomato/eGFP double positive cells decreased. In adult mice there were still approximately 3% eGFP cells also positive for tdTomato. After enalapril-mediated stimulation of the renin production in adult mice a recruitment of renin cells upstream in the afferent arteriole was visible, but there was no change of the neogenesis rate of renin-expressing cells. Interestingly, eGFP positive cells were observed in the blood, bone marrow and spleen of adult mice. A high percentage of them were positive for B cell markers.

The current data indicate that there is a pool of precursors which generates renin-expressing cells throughout nephrogenesis and persists in adulthood.
P4 Extrarenal Progenitor Cells do not Contribute to Endothelial Repair in the Mouse Kidney

Jan Sradnick¹, Song Rong², Anika Lüdemann¹, Vladimir Todorov¹, Faikah Güler², Christian Hugo¹, Bernd Hohenstein¹

¹Division of Nephrology, Department of Internal Medicine III, University Hospital CGC, Dresden; ²Division of Nephrology and Hypertension, Department of Internal Medicine, Hannover Medical School, Hannover, Germany

The role of endothelial progenitor cells for endothelial cell (EC) regeneration unclear and we previously showed that mainly endothelial colony forming cells (ECFC) can be found in kidneys after selective EC- or ischemia/reperfusion (IR) injury. Using bone marrow (BM) transplantation (BMT), we excluded the BM origin of these cells. To clarify the role of extrarenal versus BM derived cells for EC repair, we now investigated the regenerative processes of EC-IR injury following kidney transplantation (KTx) in transgenic mice with combined BMT. Ubiquitously tdTomato (tdt) expressing reporter mice served as recipients (R), C57/Bi6 mice were donors (D). A group of R also underwent BMT from D prior to renal injury, allowing us to differentiate the extrarenal niche. Reverse KTx served as control (D:tdt⁺; R:C57/Bi6). Ischemia time (25 min) was chosen to detect significant numbers of putative progenitor cells (pPC) in kidneys. On days 2 and 5, kidneys, blood and spleens were analyzed extensively using multicolor FACS-analysis and histology. pPC (CD34⁺, Flk-1⁺, CD31⁺, CD105⁺, CD146⁺, CD45⁺, CD133⁺, CD115⁺, CD14⁺), hematopoietic stem cells (c-kit⁺, Sca-1⁺, lin⁻), macrophages, dendritic, B and T cells were detected by FACS and tdt co-staining by histology.

On days 2 and 5, pPC were increased (+300 %; p<0.01). Many inflammatory cells were recruited to injured kidneys, 94% of them were tdt⁺ (extrarenal). Following KTx, tdt⁺ pPC were rarely found in blood (2 %) and spleens (8 %). In contrast, no extrarenal tdt⁺ pPC were identified in KTx kidneys by FACS or histology at any time point. pPC makers were exclusively found on renal, tdt⁻ cells. Only few tdt⁺ inflammatory cells, but no tdt⁻ pPC, were found in KTx/BMT-chimera mice.

In contrast to inflammatory cells, no extrarenal pPC are recruited to the kidney during endothelial repair. Under pathophysiological conditions endothelial repair occurs exclusively via local mechanisms that need to be specified in more detail.
P5 Immunophenotyping of Outgrowth Progenitor Cells Isolated from Membrane Oxygenators after Extracorporeal Membrane Oxygenation

Karla Lehle¹, Lucas Friedl¹, Julius Wilm¹, Alois Philipp¹, Christof Schmid¹, Matthias Lubnow²
¹University Medical Center, Department of Cardiothoracic Surgery; ²University Medical Center, Department of Internal Medicine II, Freiburg, Germany

Background: Progenitor cells were mobilized during extracorporeal membrane oxygenation (ECMO). The aim was to characterize progenitor populations accumulated on gas exchange fibres.

Methods: Adherent mononuclear cells were removed from the surface of explanted oxygenators after ECMO therapy and cultivated in endothelial cell medium on fibronectin-coated culture surfaces. Endothelial outgrowth cells and mesenchymal progenitor cells were characterized by flow cytometry using different surface markers.

Results: After 4 weeks in culture, palisading type or cobblestone type outgrowth endothelial cells (OECs) with high proliferative activity were detected. Three distinct cell populations were identified: leukocytoid cells CD45⁺/CD31⁺ (CD133⁺/VEGFR1⁺/CD90⁺/CD14⁺), endothelial-like cells CD45⁺/CD31⁺ (VEGFR1⁺/CD146⁺/CD105⁺), and mesenchymal-like cells CD45⁺/CD31⁻ (CD105⁺/CD90⁻). Endothelial-like cells formed tubular structures and did update Dil-acetylated low-density lipoprotein.

Conclusions: Endothelial and mesenchymal progenitor cells accumulated on the surface of PMP-MOs during ECMO therapy. This might be a response to critically illness or foreign surface of the ECMO circuit.
P6 Cancer Stem Cell Biomarkers and their Relation to Head and Neck Squamous Cell Carcinoma Radioresistance

Ina Kurth\textsuperscript{1,2}, Katrin Mäbert\textsuperscript{1,2}, Linda Hein\textsuperscript{1,2}, Claudia Peitzsch\textsuperscript{1,2}, Leoni A. Kunz-Schughart\textsuperscript{1,2}, Michael Baumann\textsuperscript{1,2,3}, Anna Dubrovska\textsuperscript{1,2}

\textsuperscript{1}OncoRay - National Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, TU Dresden; \textsuperscript{2}Institute of Radiooncology, Helmholtz-Zentrum Dresden-Rossendorf; \textsuperscript{3}Department of Radiation Oncology, Medical Faculty and University Hospital Carl Gustav Carus, TU Dresden, Dresden, Germany

**Background & Aim:** Current anti-cancer therapy fails to achieve long-lasting cancer cures. The development, growth, and therapy resistance of a tumor are attributed to the existence of cancer stem cells (CSCs), or tumor progenitor cells, which were discovered for many types of cancer including HNSCC. The number and properties of CSCs and their determinants of radiosensitivity are intrinsically heterogeneous and vary between individual tumor entities, which affect their radiocurability. So far it has not been clarified, if CSCs indeed play a role in radioresistance. The objective of this study is the correlation of CSC to radioresistance and their potential role in tumor relapse. The establishment of CSC biomarkers for the early detection and the specifically targeted treatment of those radioresistant CSC would essentially optimize treatment strategies.

**Materials & Methods:** In a first step we generated radioresistant cancer cell lines, which have been exposed to minimum 40 Gy given in ant fractions of 4 Gy. Comparative analysis of stem cell marker expression and assessment of \textit{in vivo} tumor growth were performed in order to elucidate putative CSC-related biomarkers with correlation to cell radioresistance. We found the aldehyde dehydrogenase (ALDH) activity as an already known cancer- and stem cell feature to be up-regulated during fractionated ionizing radiation. Thus we further investigated and characterized the ALDH positive and ALDH negative populations within the above mentioned radioresistant and non-irradiated cancer cells \textit{in vitro} and \textit{in vivo} radiobiological assays.

**Result & Conclusions:** Our study shows irradiation of cancer cells increased the tumor progenitor populations that can be defined by classical stem cell markers including ALDH activity and CD133 with increasing number of radiation fractions, which also led to activation of PI3K/AKT signaling pathway and increased expression of stem cell transcription factors like Sox2 and BMI-1. Moreover, our \textit{in vivo} studies suggest that high ALDH activity defines tumorigenic and radioresistant cell subsets by protecting cells from irradiation stress. Further comparative geneexpression analysis reveals several transcriptional regulators involved in radioresistance also known to be stem cell regulators. Thereby, our studies revealed ALDH activity as indicative for HNSCC progenitor cells with increased radioresistance and activation of the signaling pathways, which promote self-renewal. Further analysis of radioresistant and CSC overlapping signaling pathways may on one hand contribute to prediction of a tumor intrinsic radioresistance prior therapy and second help to establish new molecular targets for the development of therapeutics to use in conjunction with radiotherapy.
P7  Topotecan-induced Decrease in CD24 Expression Leads to a Loss of Tumorigenicity of Human MCF-7 Breast Cancer Cells

Stefan Huber¹, Günther Bernhardt¹, Anja Katrin Wege², Gero Brockhoff², Armin Buschauer¹

¹Institute of Pharmacy, University of Regensburg; ²Department of Gynecology and Obstetrics, University of Regensburg, Germany

Human breast cancer shows a high heterogeneity regarding the expression of proteins such as CD24, CD44 and EpCAM, which are involved in adhesion, cell migration and proliferation. The expression of these proteins, which are widely used as cancer stem cell markers, is often positively correlated with metastasis and poor prognosis. [1]

The expression of ATP-binding cassette transporters (ABC transporters) is an important mechanism of tumor resistance. Especially the breast cancer resistance protein (BCRP, ABCG2) is of great interest in particular in solid tumors, since it confers resistance to a wide variety of structurally unrelated cytostatics and defines “side populations”, an enriched source of cancer initiating cells [2].

We induced the expression of ABCG2 in MCF-7 breast cancer cells by cultivation in the presence of 500 nM of topotecan, a known substrate of ABCG2, and examined the influence on the expression of CD24, CD44 and EpCAM by flow cytometric measurements. Moreover, we investigated the influence of alterations in the expression levels of these proteins on the sphere forming ability in vitro and the tumorigenicity in immunodeficient mice.

Compared to untreated MCF-7 cells (CD24⁺/CD44⁺/EpCAM⁺) topotecan induced a shift towards lower expression levels of CD24, whereas the expression of CD44 and EpCAM remained largely unchanged. Unlike the untreated breast cancer cells, sphere formation ability and tumorigenicity after subcutaneous injection in immunodeficient (NSG) mice of the CD24⁺/CD44⁺ subpopulation was impaired. These data suggest a major role of CD24 in preservation of self-renewal capacity and tumor outgrowth and therefore might be an interesting target for new treatment approaches. Nevertheless, the therapeutic potential of anti-CD24 targeting needs to be further explored in vitro and in vivo.

P8 Radiation-induced Modulation in the Distribution of Lymphocytes in Breast Cancer Patients

Thomas Ernst Schmid¹, Eva Sage¹, Michael Sedelmayr¹, Mathias Gehrmann¹, Hans Geinitz², Gabriele Multhoff¹
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Background: Mounting evidence indicates that radiotherapy has a modulating impact on the immune system. We examined immune cells in peripheral blood of breast cancer patients undergoing radiotherapy (RT), some of which received chemotherapy (ChT) beforehand, with respect to percentage distribution.

Methods: Blood samples of 40 patients with breast cancer were collected before, at a dose of 30 Gy and at the end of RT, as well as six weeks and six months after RT. Eight of these patients received an additional, adjuvant ChT before RT. Blood samples of ten healthy volunteers were used as control. Lymphocyte subpopulations were analyzed by flow-cytometry.

Results: Our results show that ChT has a stronger affect on lymphocyte count than RT. Particularly B cells are impacted by ChT. Compared to the control (12.6±1.2 %) the number of B cells is reduced significantly to 0.8±0.2 % by ChT. There was a partial recovery of this number during RT. Regarding patients that received RT only, a significant decrease in B cell count from 11.8±0.8 % before RT to 8.0±0.7 % at the end of RT was observed. Six months after RT the percentage of B cells almost reaches control level (11.0±0.6 %). Natural killer cells were elevated after ChT (15.8±2.7 %) and decreased to normal levels (9.3±1.4 %) at the end of RT. During RT there was a transient increase in the amount of regulatory T cells of 14±2 % of the initial level, which was more pronounced without previous ChT (43±3 %).

Conclusion: Our results indicate that B and T cells are differently sensible to RT and ChT. There are also differences in the recovery time of immune cells after chemo- and radiotherapy. Different distributions of lymphocytes before, during and after RT imply a specific immune response induced by radiation. A clearer understanding of the impact of radiation and chemotherapeutic agents on immune cells and activation markers could lead towards innovative therapy concepts combining RT and ChT with immunotherapy.
Preclinical studies using xenograft tumour models have become indispensable for translational cancer research. Subcutaneous transplantation of human tumours on immunodeficient mice is well established for most experimental designs. However, orthotopic models are assumed to better mirror the clinical situation but such methods are technically more challenging regarding transplantation, radiation treatment and monitoring of treatment outcome and side effects. Our Small-Animal Image-Guided Radiotherapy (SAIGRT) platform is feasible to perform highly precise, isocentric conformal irradiation of small animals using 360° gantry rotation and imaging capabilities including fluoroscopy and cone beam CT (CBCT). Using SAIGRT and several other tools for small animal imaging we envision mimicking the clinical situation at the best possible rate. In a first step, we aim to implement different orthotopic tumour models and systematically compare their radiobiological characteristics with the heterotopic counterparts. Luciferase-expressing glioblastoma and lung carcinoma cell lines (U87MG and A549) were used for orthotopic transplantation (small pieces (< 1 mm) of subcutaneous source tumours or cell suspensions in matrigel). Tumour growth was imaged weekly via bioluminescence imaging (BLI) using IVIS Spectrum and CBCT and and the experiments were used to validate the developed image-reconstruction software. In both tumour models imaging of tumour cells injected as cell suspensions indicated multifocal metastatic-like growth while the transplantation of small tumour pieces lead to a defined tumour mass. First precise irradiation experiments with equal-weighted opposing beams (10 Gy) were performed using SAIGRT. Finally, tumours were excised for histology assessment.

The established orthotopic tumour models provide the basis to systematically compare radiobiological characteristics and parameters of the tumor microenvironment between heterotopic and orthotopic tumour xenografts. This is mandatory to further improve the selection of models for preclinical experiments.
**P10  Phase-contrast and Immunofluorescent Monitoring of HNSCC 3-D Cultures Indicate a Radiosensitizing Effect of a New Iron Chelator Drug Candidate**

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**Background & Aim:** Metabolic targeting is a promising strategy to radiosensitize resistant tumors. Thereby, pathophysiologically altered metabolic pathways and characteristics of malignant cells are therapeutically intervened. Recently, a potential for iron chelators as drug candidates in metabolic cancer targeting was discovered. We could show in an advanced in vitro assay that a novel lipophilic iron chelator inhibits cellular oxygen consumption. This appears to result in an enhanced locoregional oxygen level and reduced hypoxia, respectively, and could lead to radiosensitization. This hypothesis was to be proven.

**Materials & Methods:** Multicellular tumor spheroids of the head and neck squamous cell carcinoma (HNSCC) cell line SAS with a clear defined hypoxic fraction were applied as 3-D in vitro model. The effect of the iron chelator on the oxygen distribution was monitored by immunofluorescence using the hypoxia marker pimonidazole. The pimonidazole hypoxic fraction (pHF) was determined in frozen median spheroid sections of 10 µm thickness. The effect of iron chelator treatment in combination with external irradiation on spheroid integrity and regrowth over 60 days was analyzed with the spheroid control probability (SCP) and spheroid control dose 50 (SCD50) as analytical endpoints reflecting “cure”. Also, DNA double strand breaks upon combined treatment were studied by fluorescent staining of γH2AX foci.

**Results & Conclusion:** Exposure to the iron chelator caused a remarkable reduction of the hypoxic area in 600-650 µm SAS spheroids. Spheroid volume growth and viability of spheroid cells was not affected by short-term treatment. However, the combination with irradiation resulted in a critical reduction of the SCD50 as opposed to irradiation alone. This radiosensitizing effect was confirmed by the analysis of DNA double strand breaks.

Our data indicate that the iron chelator of interest may enhance the basic curative potential of radiotherapy. A second moderately resistant 3-D cell model has now been implemented to generalize the finding for HNSCC, and first in vivo experiments have been designed.
P11 Role of the Isocitrate Dehydrogenase 1 (IDH1) Mutation in Gliomagenesis

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Introduction: Diffuse gliomas are the most prevalent brain tumors in adults and cannot be cured. Amino-acid substitution at position 132 of cytoplasmic enzyme isocitrate dehydrogenase 1 (IDH1) is characteristic for lower grade gliomas and descendant secondary glioblastomas (GBM). The wild type IDH1 converts isocitrate to α-ketoglutarate (αKG) while reducing nicotinamide adenine dinucleotide phosphate (NADP⁺). Conversely, IDH1 R132H produces high amounts of 2-hydroxyglutarate (2HG) which leads to a hypermethylation phenotype. Nevertheless, the accumulation of 2HG cannot explain all phenomena in gliomagenesis. We aimed to gain insight into other mechanisms by which the IDH1 R132H mutation contributes to gliomagenesis.

Materials & Methods: We stably transduced an established GBM, a primary patient-derived GBM, and an immortalized astrocyte cell line with IDH1 c.395G>A cDNA using viral vectors. Transduction success was validated on DNA, RNA and protein levels using Sanger sequencing, allele-specific PCR and Western blotting. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed to measure Krebs cycle metabolite concentrations. 2HG was added to empty vector controls to detect independent mechanisms. NADP⁺/NADPH were quantified with commercial kits.

Results & Perspectives: Transduced cells carrying the mutant variant of IDH1 showed up to 1000-fold increase of 2HG in the cells as compared to empty vector controls as well as a drop in Krebs cycle metabolites downstream of isocitrate (αKG, succinate, fumarate, malate). We found about 50 % decrease in intracellular NADPH in GBM cells upon IDH1 R132H implementation but not in the respective astrocytes. However, no NADPH depletion was observed in GBM cells after exposure to 2HG. The IDH1 R132H mutation was accompanied by an essential decrease in GBM cell growth both in monolayer and 3-D spheroid culture. Thus, mutations in IDH1 lead to changes in Krebs cycle metabolites and energy homeostasis while decreasing growth rate in vitro. The analysis of IDH1 R132H-associated, 2HG-independent effects on the transcriptome level are ongoing.

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**P12 Overexpression of Survivin Causes Aneuploidy, Defective Mitosis and DNA Damage in Glioma Cells**

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**Introduction:** Survivin, a protein that belongs to the inhibitor of apoptosis proteins (IAPs) family, is highly expressed in cancer cells but is almost absent in normal tissue. Actually, it was shown that Survivin is one of the four most highly expressed genes found in tumors. So far it is assumed that Survivin’s IAP function drives tumor progression and confers resistance against standard therapies. Besides its role and function in apoptosis, Survivin forms during mitosis the chromosomal passenger complex (CPC) together with its partners Borealin, inner centromere protein (INCENP) and the enzymatically active member Aurora B kinase. The function of this complex is to sense and correct non-bipolar microtubule-kinetochore interactions, regulating chromosome segregation and cytokinesis. Survivin’s main molecular function in particular is linked to the control of the spindle assembly checkpoint and cytokinesis. Recently, we have shown that Survivin RNAi affects chromosome segregation and DNA integrity emphasizing its role as a chromosomal passenger complex protein (CPP). Despite this important role, the influence of its overexpression on chromosome instability has not been addressed so far. The aim of this study was therefore to elucidate the consequences of Survivin’s overexpression as a CPP on chromosomal instability and aneuploidy thereby driving tumor progression.

**Methods:** Glioma cell lines (U373-MG, U87-MG and U87shp53) were transduced with retroviral vectors encoding Survivin-myc-HA. As controls, cells transduced with an empty vector (mock) were used. 72 hours after transduction, cells were subjected to flow cytometry, apoptosis assays, polyploidy analysis, DNA damage analysis, Western Blot analysis, confocal microscopy and spectral karyotyping (SKY) analysis.

**Results:** The exogenous overexpression of Survivin caused a significant increase in the fraction of polyploid U373-MG cells and U87shp53 cells when compared to mock-transduced controls. However, there was no increase observed in the fraction of polyploid cells in U87-MG wild type cells with ectopic overexpression of Survivin-myc-HA. BrdU incorporation analysis showed that cell proliferation was not affected in any of the cell lines. The overexpression of Survivin also resulted in mitotic defects which were revealed by the appearance of extra and lagging chromosomes, chromosome misalignment and chromosomal bridges. Nuclear atypia and pleomorphy, including pleomorphic nuclei and abnormal interphase cells, were also observed. Furthermore, Western blotting and confocal laser scanning analysis showed that cells overexpressing Survivin suffered from DNA damage. Finally, SKY analysis showed a highly significant increase in the number of structural chromosomal aberrations when compared to control cells.

**Conclusion:** Our results suggest that deregulated Survivin expression levels contribute to chromosomal instability, and may lead to tumor progression. An understanding of the biological mechanisms that account for aneuploidy could lead to useful insights with respect to novel anti-cancer therapeutic approaches in glioma.
POSTER ABSTRACTS

P13 Chemical Inhibition of Mitotic Aurora B Causes Chromosomal Instability in Tumor Cells and Exerts Antitumoral Effects Irrespective of p53 Status

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The „Chromosomal Passenger Complex“ (CPC) is one of the key regulators of cell division involved in the coordination of chromosomal and cytoskeletal events. The enzymatically active member of the complex is Aurora B kinase which has multiple roles during mitosis such as directing chromosome condensation, regulating kinetochore-microtubule attachment, enabling the mitotic spindle checkpoint, and triggering the completion of cytokinesis. Targeting Aurora B by RNAi or chemical inhibition overrides the spindle checkpoint and drives cells through an aberrant mitosis, followed by endoreduplication and eventual cell death. Therefore Aurora B represents a promising target for anti-cancer therapy.

In this study, we evaluated the biological effects of pharmacological Aurora B inhibition and the role of the tumor suppressor protein p53 in the cell cycle regulation and chromosomal segregation in HCT116, U87-MG and primary HT7606 glioma cells. HCT116wt, HCT116p53-/-, U87-MGwt, U87-MGshp53 and primary HT7606 glioma cells were incubated with medium containing the specific Aurora B inhibitor AZD 1152-hQPA (barasertib-hQPA) or DMSO, respectively. Subsequently, cells were subjected to Western blot-, flow cytometry-, immunofluorescence analysis and Chromium release assay.

Interfering with Aurora B function caused in all tested cell lines a heavily impaired cytokinesis resulting in polyploidy, disturbed mitosis and merotelic kinetochore spindle assemblies, irrespective of the p53 status. Cells with a functional p53 protein showed p53 and p21waf/cip activation after incubation with Aurora B inhibitor. A detailed Flow Cytometry analysis revealed a correlation between the increased p21waf/cip expression and increasing DNA content in wild type cells with Aurora B inhibition compared to p53-deficient cells. Conversely, BrdU incorporation in wild type cells treated with Aurora B inhibitor decreased with DNA content compared to p53-deficient cells. Nonetheless, polyploid wild type cells even with a DNA content larger 4n were still able to incorporate BrdU, indicating a p53/p21waf/cip mediated transient G1 arrest after Aurora B inhibition. Furthermore, the inhibition of Aurora B induced in U87-MG and HCT116 cells a DNA damage response as indicated by γH2AX, activated ATM and CHK2 kinases and p53 phosphorylation. Additionally, interfering with Aurora B function caused in all tested cell lines cell death which was augmented in p53-deficient cells and a significantly reduced clonal survival. The death receptor TRAIL R2/DR2 was strongly upregulated in Aurora B treated cells, inducing an enhanced cytotoxic response of allogenic human nk cells.

In conclusion, our results highlight the important role of Aurora B as a chromosomal passenger protein in controlling chromosomal segregation by regulating bipolar spindle attachment and cytokinesis. Furthermore, chemical inhibition of Aurora B kinase severely affects proliferation and survival of tumor cells irrespective of p53 status. Therefore, therapies targeting Aurora B kinase with a concomitant immunotherapy might represent a promising avenue for adjuvant local treatment of gliomas.
The most frequent brain tumour in adults, glioblastoma multiforme (GBM), is despite intensive treatment currently not curable. Our research is focused on the development of individualized therapies which might overcome the treatment resistance of GBM. The established GBM cell lines U87MG, U251MG, U138MG, LN229, A7 and HGL21 were characterized in relation to their radio-responsiveness to X-rays \textit{in vitro}. The surviving fraction after irradiation with 2 Gy varied between 55\% (U87) and 85\% (A7) indicating strong radio-resistance of the cell lines. The existence of cancer stem cell (CSC) subpopulations could promote the radio-resistance. The \textit{in vitro} CSC-populations were evaluated by immunohistochemical (IHC) staining for the biomarkers Nestin, SOX2 and CD95. The CSC-markers showed distinct differences in their staining patterns but CSC and non-CSC populations could not be discriminated. A possible strategy to increase treatment response is the deprivation of the essential amino acid methionine (M). Therefore the cell lines were subjected to six different M concentrations (101.0 (regular medium) - 0.0 µM) over five days followed by a three day recovery phase. Five out of six cell lines showed reduced plating efficiencies in response. The combinational effect of the diet followed by radiation treatment was additive except in cell line LN229 where a synergistic effect of M depletion and radiation was observed. The cell lines were established as xenograft tumours in NMRI nude mice to correlate the \textit{in vitro} data with tumour control dose experiments (TCD\textsubscript{50}), limited dilution assays (TD\textsubscript{50}) and M-deprivation experiments. Preliminary results indicate that the TD\textsubscript{50} differ substantially among the cell lines. In contrast to \textit{in vitro} growing cell lines, excised tumours showed distinct positive subpopulations for CSC markers.
P15  Arginine Deprivation: Treatment Option for Non-auxotrophic Glioblastomas?

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Background & Aim: Arginine withdrawal effectively inhibits tumor growth of arginine-auxotrophic cancers in vitro and in vivo. However, our group showed that even non-auxotrophic cancer cells in 3-D multicellular spheroid culture (MCTS) are growth-inhibited and may be radiosensitized by lack of arginine. Enzymotherapeutic arginine deprivation is a systemic approach and could thus be an option for the treatment of therapeutically challenging, highly invasive glioblastomas. To prove this hypothesis, we extended our 2-D in vitro study presented last year to more relevant 3-D assays reflecting tumor cell growth and invasion.

Materials & Methods: U87-MG, U87-MG-shp53 (p53-wt and -knockdown) and U251-MG (p53-mutant) eGFP-transduced cells were applied in a first approach. 3-D MCTS were cultured in liquid overlay using a standardized semi-automated set-up. Arginine deprivation was realized by transfer of MCTS into arginine-free medium supplemented with dialysed serum or by treatment with recombinant human arginase (rh-Arg). Upon treatment, viable cell numbers of 2-D monolayer cultures were determined, spheroid volume was monitored by phase contrast imaging and invasion distance of spheroid cells in a 3-D collagen-I invasion assay was evaluated by fluorescence life imaging.

Result & Conclusions: All three glioblastoma cell lines formed MCTS, but U251-MG cells failed to grow in the 3-D environment. In contrast, all MCTS including U251-MG invaded into collagen-I gel. Here, the p53-knockdown/ mutant MCTS appeared to be less invasive than the p53-wt cultures. According to the monolayer experiments, growing MCTS completely lost their proliferative activity under acute arginine withdrawal, independent of the p53-status. Furthermore, invasion capacity of U87-MG-wt MCTS was reduced during arginine starvation. In summary, arginine deprivation showed a clear therapeutic potential in the 3-D assays. However, before turning into whole animal studies, the approach shall be applied in primary glioblastoma cultures, in combination with standard treatments for glioblastoma patients, and taking into account in vivo environmental conditions, e.g. the locoregional presence of citrulline.

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P16  Citrulline Concentration as a New Pitfall for the Arginine Deprivation-based Therapy

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Background & Aim: Arginine deprivation is a promising strategy to sensitize cancer cells to conventional therapies. Arginine can be depleted by arginine-degrading enzymes injected into patients’ blood stream. Two enzymes, bacterial arginine deiminase (ADI) and recombinant human arginase I (ARGase), are currently in clinical trials. However, tumors may utilize citrulline, but not ornithine, for arginine synthesis during starvation. We were the first to show that the amount of citrulline is critical for the radiosensitization effect of arginine deprivation. The aim of this study was to unravel the impact of citrulline on cell cycle progression under arginine limitation.

Methods: HT29 human colorectal cancer cells, which acquire the ability to utilize citrulline for arginine synthesis upon starvation, were grown in exponential monolayer culture. Arginine-free DMEM was supplemented with either arginine or citrulline at concentrations according to medium (400 µM) or human blood (50 µM). Cell viability was assessed by cell counting and MTT assay. For dynamic cell cycle analysis, cells were pulse-labeled with 5-ethynyl-2-deoxyuridine, incubated in the media of interest for another 0-8 hours, and measured by flow cytometry.

Results & Conclusion: Citrulline at concentrations equimolar to arginine in DMEM partly compensated for the growth retardation induced by arginine-withdrawal. 50 µM citrulline did not support cell proliferation even if the cells were fed every 24 hours with fresh citrulline-containing arginine-free medium. Subsequent dynamic cell cycle analysis revealed that the S-phase was prolonged at 50 µM arginine (~10 hours) as compared to 400 µM arginine (~8 hours). Cells prevailed in S phase for ~11 hours at 400 µM citrulline but failed to progress through the cell cycle when exposed to 50 µM citrulline. These data were supported by Western blot analyses of proteins involved in cell cycle regulation. Taken together, our data suggest that the amount of citrulline during arginine withdrawal is crucial for cell cycle progression.

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P17 Metabolic Targeting of a Semi-essential Amino Acid: The Challenge to Identify Responder and Non-responder HNSCC Cells

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Background & Aim: Arginine auxotrophic tumor cells which lack the rate-limiting enzyme argininosuccinate synthetase (ASS) for arginine de novo synthesis from its precursor citrulline, show high response rates to arginine deprivation monotherapy in clinical trials phase I/II. However, first in vitro studies imply that the treatment outcome, i.e. after irradiation, could also be improved in selected ASS-positive tumors by arginine deposition. Radiotherapy (RT) is a standard treatment option for advanced and recurrent head and neck squamous cell carcinomas (HNSCC). But it is still challenging to achieve tumor cure. We therefore study a panel of HNSCC cell lines to identify and better characterize HNSCC responders and non-responders to radiosensitization by arginine deprivation.

Materials & Methods: A panel of nine HNSCC cell lines is implemented in the study. Arginine deprivation is achieved by using arginine-free DMEM with dialyzed serum; citrulline is supplemented in physiological concentrations for mechanistic insight. Monolayer cell growth and regrowth are assessed by cell counting, and spheroid volume growth is monitored by phase contrast imaging. Arginine deprivation is combined with single-dose X-ray, and radiosensitization capacity is initially determined by colony formation assays. ASS protein expression is determined by immunoblotting analysis.

Results & Conclusion: All HNSCC cell lines express ASS protein at different intrinsic levels. Independent of that, acute arginine deprivation leads to cell growth retardation or even to a complete growth arrest in 2-D cultures. The regeneration capacity after starvation is reduced dependent on the time of starvation and in a cell-line specific manner. Physiological citrulline concentration can not totally compensate the effect of arginine depletion. The heterogeneous growth capacity neither clearly correlates with the ASS protein expression profile nor with the radiosensitizing effect of arginine deprivation found in some cell lines. Experiments are ongoing to gain deeper insight into the underling mechanism and to better discriminate responder and non-responder HNSCC cells with respect to radiosensitization.

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**P18  Eye the Putative Anticancer Potential of the Natural Food Compound Methylglyoxal (MGO)**

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**Background & Aim**

MGO is a natural food compound mainly produced by the Maillard reaction between reducing sugars and proteins. MGO showed anti-cancer effects in several publications but head and neck cancer (HNSCCs) cells were not implemented. We intended to evaluate the impact of MGO on HNSCC cell behavior and radioresponse as we hypothesized a radiosensitizing potential - beneficial for the treatment of HNSCC patients. Our first observations indicated that this highly reactive α,β-dicarbonyl might be quite instable. This was not considered in earlier studies and our rationale to monitor MGO as a function of time in cell culture medium and blood.

**Materials & Methods**

Cell viability and growth were analyzed in SAS (HNSCC) monolayer cultures via MTT-assay, cell counting and colony formation assay. The impact on three-dimensional growth kinetics was recorded by semi-automated assessment of spheroid diameter and volume. MGO degradation in medium and blood was measured by HPLC after derivatization with o-Phenyldiamine. Amino acid analysis in cell culture media before and after MGO incubation was performed to quantify the conversion rate with its main reaction partners.

**Results & Conclusion**

There is clear evidence that SAS cells are quite sensitive and might be radiosensitized by MGO exposure. However, the heterogeneity of effective doses in the different experimental systems applied indicated low MGO stability and a half-life affected by both the cells and their environment. This was proven in a collaborative approach of cell culture, laboratory medicine and chemical-analytical departments. We found MGO to be a difficult manageable compound for future in vivo administration if it cannot be sufficiently stabilized as seen for example in some honeys. Alternative metabolic targeting concepts to take advantage of the MGO anti-cancer efficacy are thus considered and will be discussed.
P19 Detection and Characterization of Circulating Epithelial Tumor Cells in Patients with Hepatocellular Carcinoma (HCC)

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Introduction: Hepatocellular carcinoma is the main primary tumor entity of the liver characterized by a high rate of recurrence and metastases. In this context disseminated circulating epithelial tumor cells (CETCs) are discussed to represent a cell population with a promising diagnostic and prognostic potential. The aim of the study was the establishment of a method for the identification of those cells via suitable cell surface markers.

Methods: Cells of the HCC cell lines HepG2 and Hep3B were cultivated in RPMI 1640 with 10 % FCS. In line with the conflicting discussion of appropriate CETC markers we investigated the expression of three different cell surface proteins: epithelial cell adhesion molecule (EpCAM), epithelial glycoprotein mucin 1 (MUC 1) and asialoglycoprotein receptor 1 (ASGPR 1). In an initial experimental setting specificity of the selected antigens was verified by immunohistochemistry and FACS analysis. Then, blood samples from 60 HCC patients scheduled for liver transplantation were analyzed for the occurrence of epithelial antigen positive cells. Further investigations on gene expression level were carried out via a suitable single cell preparation technique based on an automated system using a glass capillary. The prepared single cells were analysed by a very sensitive and specific qPCR regarding the expression of a selected gene set.

Results: ASGPR 1, MUC 1 and EpCAM proteins could be detected on cell line cells as well as in formalin-fixed, paraffin-embedded HCC tissue sections showing characteristic staining intensity and distribution. Analysis of peripheral blood samples from HCC patients also revealed the occurrence of cells expressing one or more of the investigated proteins specifically. EpCAM turned out to be the most reliable marker (detectable in 86 % of all samples) with a broad and patient specific range between 400 and 140,000 cells per ml blood. In order to further study EpCAM-positive cells a single CETC preparation method was established. Cell viability and biological activity was characterized by propidium iodide staining and expression analyses of housekeeping genes.

Conclusion: The determination of EpCAM positive cells in the peripheral blood of HCC patients can contribute to the evaluation of prognosis, risk of recurrence and therapy response. The established methods represent the basis for further investigations on single cells regarding the discovery of further specific biomarkers.
P20  Heterogeneity of Brain Metastases Derived from Melanoma

Beate Rinner¹, Katharina Meditz¹, Marie-Therese Frisch¹, Sabrina Riedl², Helmut Schaider³, Bernadette Liegl-Atzwanger⁴, Karl Lohner², Dagmar Zweytick²

→ Abstract s. Session 7

P21  Real-Time Deformability Cytometry: High-Throughput Mechanical Phenotyping for Marker-free Cell Functional Assays

Oliver Otto¹, Philipp Rosendahl¹, Alexander Mietke¹, Stefan Golfier¹, Angela Jacobi¹, Christoph Herold¹, Nicole Töpfner¹, Jochen Guck¹,²

→ Abstract s. Session 2

P22  Picoliter Bioreactors: Learning from Single Cells about Large-Scale Bioprocesses

Alexander Grünberger, Christopher Probst, Wolfgang Wiechert, Dietrich Kohlheyer

→ Abstract s. Session 7
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Acknowledgement: Chromocyte is free to use due to the invaluable financial support of our Corporate Sponsors.
Fluorescence light microscopy is of widespread use to study biological systems. Appropriate fluorescence dyes or labeling tags give scientists the ability to specifically visualize cells, cell organelles, proteins or other molecules of interest. However, in relation to the chemical impact accompanied with the labeling agents and procedure recent trends in cell analytics favor the use of label free methods. So it is possible to analyze interactions of cell compartments of interest close to \textit{in vivo} conditions without labeling-induced chemical alteration. It is well known that most organic molecules are able to emit fluorescence light after an excitation with light at a characteristic wavelength. Though, the fluorescence quantum yield is significantly reduced by different quenching effects and strongly affected by the temperature of the specimen and its environment. Normally it’s possible to reduce such quenching effects and increase the fluorescence quantum yield by decreasing the temperature of the sample.

In this context a combined system for laser induced fluorescence spectroscopic and microscopic measurements at temperatures down to 20 K has been developed, characterized by a commercial confocal laser scanning microscope, a very sensitive spectroscopic detection system, and a special cryogenic measuring cell. The cryogenic measuring cell is characterized by a closed cryogen cycle. Moreover, it can easily be adapted to common commercial light microscopes without time consuming reconfigurations. Microscopic measurements with an up to 630-fold magnification, temperature control in the range between 320 K and 20 K by steps at 1 K and an easy sample handling in x/y dimensions are possible.

Benefits of the novel cryo microscopic fluorescence technique are shown in different representative investigations.

- Several microscopic experiments demonstrated an up to 100-fold improved sensitivity in detection of fluorescent dyes at cryogenic conditions compared to room temperature.
- A substantially lower photobleaching of the fluorophores was observed at 20 K compared to room temperature.
- Laser-induced cryogenic fluorescence microscopy allowed visualization of novel compounds inside of cells without additional chemical modification, e.g., by coupling fluorophore substituents.

The results show that the new cryogenic measuring chamber represents a powerful and gainful tool for fluorescence based investigations. They illustrate a big potential in identifying further application areas.
More than a billion years of evolution have resulted in the development of a variety of fungi that produce a vast diversity of enzymes, many of which have potential biotechnological applications. A submerged cultivation of the fungi is possible, but the full potential, particularly of basidiomycetes, is not exploited under these conditions. Solid-state fermentation (SSF) is a robust process that is well suited to the on-site cultivation of basidiomycetes that produce enzymes for the treatment of lignocellulosics and consequently for the production of 2nd generation bioethanol. However, optimized cultivation and process conditions are required to produce sufficient quantities of material for these purposes. One of the key variables that must be monitored during any fungal fermentation process is the biomass concentration, which is used to characterize fungal growth and its kinetics. However, direct biomass determination is not possible during SSF because the fungi grow into the substrate and use it as a nutrient source. This necessitates the use of indirect methods that are either very laborious and time-consuming or can only provide biomass measurements during certain growth periods. Indirect biomass measurements can be obtained using a range of different techniques such as the measurement of cell-specific components or biological activity or the quantification using physical and optical methods.

Here we describe the development and optimization of a new rapid method for fungal biomass determination during SSF that is based on counting fungal nuclei by flow cytometry. Fungi were grown on malt extract agar plates or an organic substrate (mixture of corn silage and pine wood chips) and its concentration was measured by isolating the nuclei from the fungal hyphae after cell disruption, staining them with SYTOX® Green, and then counting them using a flow cytometer. A calibration curve relating the dry biomass of the samples to their concentrations of nuclei was established. Multiple buffers and disruption methods, like milling, ultrasonic and microwave treatment, were tested. The results obtained were compared to values determined using the method of ergosterol determination, a classical technique for fungal biomass measurement during SSF. Our new approach was successfully used to measure fungal biomass on a range of different scales, from 15 mL cultures to a laboratory reactor with a working volume of 10 L (developed by the Research Center for Medical Technology and Biotechnology (fzmb GmbH)) and can be also used in industrial applications.
Cytometric Fingerprinting for Microbiome Diagnostics

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¹Helmholtz-Zentrum für Umweltforschung - UFZ, Germany; ²Stockholm Resilience Centre, Stockholm University, Sweden

The activity of natural microbial communities, i.e. microbiomes, is of high importance for numerous managed or engineered industrial systems (e.g. wastewater treatment, anaerobic digestion). These microbiomes, their dynamics, and the role of their complexity for system functions are only superficially understood. The bioreactors are usually operated based on bulk parameters and empirical expert knowledge. Thus, the microbiomes remain a “black box”. Flow cytometry is a high-throughput single cell based method with high potential for routine microbiome diagnostics. Using a MoFlo cell sorter morphological cell features can be analyzed by forward scattering behaviour (FSC) and the DNA content, measured using the AT specific fluorescent dye DAPI. Cytometric analyses of these parameters result in distinct, fingerprint like, patterns. These cytometric fingerprints are highly reproducible and represent the structure of the microbial community at a certain point of time. Structure-function-relationships can be revealed based on correlations between the microbial community structure, dynamics, and the system performance.

The challenge is the detailed and standardized analysis of these fingerprints. In the recent years four different principles for cytometric fingerprint analysis were used:

1) Dalmation plot analysis
2) Cytometric Histogram Image Comparison (CHIC)
3) Cytometric Barcoding (CyBar)
4) FlowFP

The four methods differ regarding the required experience in handling of cytometric data sets, detection level of changes, time demand, and software application. They were systematically compared and tested for their applicability (Koch et al., 2014). The workflow of the four tools is presented and packages for their direct application can be downloaded.

P27  Plasmid Copy Number Variation in Pseudomonas putida Analysed by Cell Sorting and Digital Droplet PCR

Michael Jahn¹, Carsten Vorpahl¹, Dominique Türkowsky¹, Martin Lindmeyer², Bruno Bühler², Hauke Harms¹, Susann Müller¹
→ Abstract s. Session 10

P28  Flow Cytometry for Energy Balances of Phytoplankton Organisms

Susanne Dunker, Torsten Jakob, Christian Wilhelm
→ Abstract s. Session 10

P29  Enhanced Viability of Microalgal Populations by Photoperiodic Cycles

Felix Krujatz, Thomas Bley, Jost Weber
→ Abstract s. Session 10

P30  Bioinformatical Approaches for the Analysis of Complex Microbial Communities

Joachim Schumann¹, Christin Koch¹, Susanne Günther¹, Ingo Fetzer², Susann Müller¹
→ Abstract s. Session 8

P31  Staphylococcus aureus Infection Induces Human T Cell Apoptosis and Increased Mortality in Humanized Mice

Janin Knop¹, Frank Hanses², Nancie Archin³, Joachim Gläsner⁴, Andre Gessner⁴, Anja Kathrin Wege¹
→ Abstract s. Session 10
P32 Memory CD4 T Cells in Murine Bone Marrow are Resting in Terms of Migration

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We have previously identified a population of memory CD4 T cells which preferentially reside in the bone marrow (BM) of mice docked onto IL-7 producing stromal cells. While these cells are resting in terms of proliferation and transcription, after activation, they are potent cytokine producers and support the production of high affinity antibodies, proving their superior memory cell capacity over their peripheral counterparts. The objective of this study was to examine the migratory potential of memory CD4 T cells in the bone marrow of mice in order to determine whether they can be further classified as resting in terms of migration.

The formation of polymerized F-actin at the leading edge of a cell after chemokine stimulation is a critical step in the migration process. Ex vivo analysis of BM memory CD4 T cells showed no polarization and reduced overall content in comparison to peripheral memory T cells, suggesting that their cytoskeleton was not activated in situ. Furthermore Tiam1, a key Rac1 activator, which is known to be important for chemokine mediated migration was also reduced. This translated into a reduced ability of BM CD4 memory T cells to respond to chemokines in vitro. Furthermore, BM T cells survived extremely poorly ex vivo, suggesting that their BM stromal cell niche is essential for their long-term survival in vivo.

Overall, bone marrow memory CD4 T cells appear to have a resting phenotype in relation to the migration mediators tested. In this resting state, they lack the necessary receptors required to leave the bone marrow and this immobility may ensure that the T cells remain attached to their stromal niche. Ongoing studies will establish whether after activation, BM memory T cells re-gain their mobility in order to leave the bone marrow and provide efficient help to B cells in the periphery.
FAT10 is the only ubiquitin-like modifier which directly targets hundreds of substrate proteins for rapid degradation by the proteasome. FAT10 consists of two ubiquitin-like domains which are joined by a short linker. Via a diglycine motif at its C-terminus FAT10 is first activated by the E1 enzyme UBA6 and then transferred to the E2 enzyme USE1 in order to become conjugated to its substrates. Docking of FAT10 to the 26S proteasome occurs via the VWA domain of Rpn10 (S5a). While the conjugation and proteasomal targeting of FAT10 are fairly well understood, the biological functions of FAT10 have remained elusive. FAT10 deficient mice are normal except for a hypersensitivity to lipopolysaccharide. This phenotype as well as the strong synergistic inducibility of FAT10 by the pro-inflammatory cytokines interferon-gamma and tumor necrosis factor-alpha point at a role of FAT10 in the immune system. Consistent with this notion, the fat10 gene is localized in the major histocompatibility complex (MHC) and the fat10 mRNA is most prominently expressed in thymus, lymph nodes and spleen. Previously, we have shown that the fusion of FAT10 to the long lived nucleoprotein (NP) of lymphocytic choriomeningitis virus leads to its accelerated degradation by the proteasome. This enabled an enhanced presentation of NP-derived peptides on MHC class I molecules and a more potent stimulation of NP-specific cytotoxic T lymphocytes. The latter result inspired us to hypothesize that FAT10 may influence the T cell repertoire. Here we show that FAT10 in expression can be assigned to medullary thymic epithelial cells. These cells play an essential role in negative selection and central tolerance induction as they present self-antigens to developing thymocytes and promote elimination of self-reactive T cells. Furthermore, by analysis of several T cell receptor transgenic mouse strains which were proficient or deficient for FAT10 we could show that FAT10 seems to have an epitope dependent effect on thymic selection. Taken together, FAT10 was identified as a novel player in thymic selection.
P34  Heterogeneous T Cell Reactivity Against Microbiota in Patients with Crohn´s Disease

Petra Bacher¹, Jochen Maul², Uta Syrbe², Britta Siegmund², Alexander Scheffold¹,³
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It is assumed that inflammatory bowel diseases (IBD) might result from an inappropriate inflammatory response against intestinal microbiota. However, due to the technical limitations and the complexity of the microbiota, direct evidence for aberrant immune responses against microbiota in humans is scarce. In particular the role of fungal intestinal commensals in health and disease is currently unclear. We applied a recently developed highly sensitive technology for the detection of rare antigen-reactive CD4⁺ T cells (Antigen-reactive T cell enrichment, ARTE), to show that specific T cell responses against intestinal bacteria and the fungi (*Candida albicans*) are strongly increased in peripheral blood of Crohn´s disease (CD), but not ulcerative colitis patients. The increased reactivity is transient and associated with high CRP levels, indicating a direct parameter for increased gut permeability and disturbed barrier function. Further multi-parameter characterization of *C. albicans*-reactive T cells revealed altered cytokine expression patterns. These patterns were stable in individual patients (up to 1 year) but diverse between different patients. In fact, classification of CD patients according to the *C. albicans* specific cytokine pattern correlated with disease behavior and localization, providing direct evidence that microbiota-specific T cell differentiation may affect the disease outcome. Thus antigen-specific T cell responses against intestinal microbiota are specifically modulated in CD patients. Furthermore, our data reveal that microbiota-specific T cell reactivity patterns are heterogeneous but stable and may provide a new diagnostic or prognostic option for this complex disease.
P35 Human Memory T Cells From the Bone Marrow are Resting and Maintain Long-Lasting Memory

Anna Okhrimenko¹, Joachim R. Grün², Kerstin Westendorf¹, Zhuo Fang³, Simon Reinke⁴, Philipp von Roth⁵, Georgi Wassilew⁵, Anja A. Kühl⁶, Robert Kudernatsch⁷, Sonya Demski⁷, Carmen Scheibenbogen⁷, Koji Tokoyoda⁸, Mairi A. McGrath¹, Martin Raftery⁹, Günther Schönrich⁹, Alessandro Serra¹, Hyun-Dong Chang¹, Andreas Radbruch¹, Jun Dong¹

→ Abstract s. Session 8

P36 Memory CD8⁺ T Cells Colocalize to IL-7⁺ Stromal Cells in Bone Marrow and Rest in Terms of Proliferation and Transcription

Özen Sercan Alp¹, Sibel Durlanik², Joachim Grün¹, Mairi McGrath¹, Daniel Schulz¹, Marcus Bardua¹, Koichi Ikuta¹, Fritz Melchers³, Rene Riedel¹, Sandra Zehentmeier¹, Anja E. Hauser¹, Koji Tokoyoda¹, Hyun-Dong Chang¹, Andreas Thiel², Andreas Radbruch¹

→ Abstract s. Session 3

P37 Function of IRAG in Murine T-Lymphocyte Development

Johannes Philip Huettner¹, Anja Kathrin Wege², Jens Schlossmann¹

→ Abstract s. Session 11

P38 B7 Costimulation and Intracellular Indoleamine 2,3-Dioxygenase (IDO) Expression in Umbilical Cord Blood and Adult Peripheral Blood

Gergely Toldi, Enikő Grozdics, László Berta, Tivadar Tulassay

→ Abstract s. Session 12
The novel mass cytometry technology allows simultaneous analysis of more than 30 parameters on single cells. The present panel was developed for a comprehensive immunophenotyping of human blood samples to monitor immune responses in rheumatic patients treated with anti-inflammatory biologicals. The aim of the present study was to develop a 29 marker (31 multi-parameter) panel for mass cytometric analysis of major and minor leukocyte populations including their differentiation-dependent subsets and appropriate activation marker molecules in whole blood samples depleted of erythrocytes. Here, we present a multi-parametric panel design strategy for mass cytometric analysis with subsequent unsupervised cluster analysis and visualization of the data. The strategy involves all major aspects of quality control including the validation of metal-conjugated antibodies and optimization to receive the highest signal-to noise ratio for chosen target molecules with respect to the mass response of the individual CyTOF instrument. Additionally, spillover assessment of possible background signals from different sources into target mass channels was performed for each marker used in the CyTOF panel. The CyTOF protocol was tested by comparative measurement of blood samples from healthy individuals and systemic lupus erythematosus patients in parallel using conventional multicolor cytometry. It could be shown that there is high accordance between both technologies as long as protocol establishment is thoroughly controlled. Furthermore, CyTOF data were analyzed by the automated cluster analysis immunoClust as well as a manual gating procedure. Frequencies of cell subsets are in high accordance when comparing both approaches. The CyTOF-panel presented can be used as a core panel for monitoring immune responses in different fields of medical research and can be modified by adding or substituting antibodies of interest.
P40 Expression and Localisation of Mechanotransducers YAP and TAZ in Aortic Valvular Interstitial Cells – Exploring the Role of YAP/TAZ for the Development of Stenotic Aortic Valves

Claudia Dittfeld, Wolfgang Witt, Anett Jannasch, Janin Andres, Katrin Plötzke, Maria Feilmeier, Nadine Lange, Klaus Matschke, Thomas Waldow
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Background & Aim: Processes of mechanotransduction and its molecular players are of great relevance for the development and progression of stenotic aortic valves. For the year 2015 the prognostic incidence in the EU, Switzerland, Japan and North America to develop a valvular aortic sclerosis is 35 % resulting in a total frequency of 3 % of people ≥ 65 years old who develop a valvular aortic stenosis with the indication for surgical replacement of the valve. Stiffening of valve tissue, fibrosis and calcification of leaflets accompany the development of the disease resulting in restricted functionality. Recently the non-canonical regulation of transcriptional co-factors YAP (Yes-associated protein) and TAZ (PDZ-binding motive, WWTR1) have been defined as mediators and regulators of mechanotransduction e.g. in cancer cells, mesenchymal stem cells and in mammary epithelial cells. Nuclear localisation and therewith activation of YAP/TAZ are related to matrix stiffness and composition, cell density and stretching of the cells. Rho activity and the actomyosin cytoskeleton are required for activation of co-factors. Aim of the project is to define the relevance of YAP/TAZ in mechanotransductional regulation of aortic valve disease by investigating valvular interstitial cells responsible for maintenance of extracellular matrix of the aortic valve.

Materials & Methods: Porcine (pVIC) and human (hVIC) aortic valvular interstitial cells were isolated by enzymatic digestion using collagenase. Cells were cultured on collagen coated cell culture plates for up to five passages. To investigate impact on matrix stiffness and cell density, hydrogel coated cell culture plates reflecting fibrotic and healthy aortic heart valve tissue at different cell seeding numbers were used and applied. Total protein expression was determined by Western Blotting. Immunofluorescence staining of YAP/TAZ expression in cells was performed to investigate cellular localization after acetone fixation of cells cultured on slides. To determine effects of stretch, Flexercell-Tension system was used, plating VICs on flexible culture plates. Cover slides with hydrogel coating exhibiting different stiffnesses for immunofluorescence staining, methods for quantitative mRNA expression analysis and determination of splice variants are currently established.
Results: Total TAZ protein expression is significantly upregulated in hVICs and pVICs in cells cultured on stiff cell culture plastic in confluent and exponentially growing culture compared to cells growing on hydrogel coated plates with the elastic moduli of 8 and 25 kPa. YAP protein expression is lower in confluent hVIC culture but not in exponentially growing cells at 8 and 25 kPa. Also there is no significant difference of YAP protein in pVIC cells in both cell densities. The decrease of expression was independent from the stiffnesses of 8 and 25 kPa. Immunofluorescence staining revealed two different observations using different antibodies. On the one hand YAP/TAZ is localized in the nuclei and the cytoplasm of h/pVICs on stiff glass slides, on the other hand small filaments were detected after staining with a second antibody that exhibit a partially co-localisation to focal adhesion kinase. Preliminary results indicate the reduction of nuclear localisation after cell stretch.

Conclusions: Due to the upregulation of YAP/TAZ expression in VICs cultured on stiff cell culture plastic compared to cultures on soft hydrogel coated plates and the nuclear localization of the proteins, it is hypothesized that transcriptional co-activators YAP and TAZ are involved in processes of mechanotransduction in hVICs. YAP splice variants, interaction partners of YAP/TAZ as well as cellular localisation of these proteins on soft matrices and/or after stretching of the cells are focussed on in further experimental work.
P41 Absolute Cell Counting of Gram-positive *S. aureus* Using Flow Cytometry

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The determination of absolute cell numbers in bacterial cultures is usually accomplished by counting the colony forming units (CFU). After preparation of serial dilutions bacteria are plated on agar plates and incubated at the appropriate temperature. However, the process is inaccurate, time- and material-extensive and can only be applied to cultivable organisms.

Looking for an alternative method to reliably count bacterial cells for infection experiments, we employed the bench top 2 laser flow cytometer Guava easyCyte 6-2L (Merck Millipore). This flow cytometer allows absolute cell counting and concentration determination without the use of counting beads. To distinguish the bacteria from background it is necessary to stain them with a fluorescent dye. In this work, we tested three different dyes which were all excited with the 488 nm laser and the fluorescence was detected in the GRN channel (filter 510-540nm): i) Syto9 dye which is known to stain DNA as well as RNA and to emit fluorescence after binding to either one when excited. ii) The carboxyfluorescein diacetate succinimidylester, CFDA-SE, is a cell permeable dye generally employed in animal cell proliferation research and iii) vancomycin BODIPY® FL conjugate which contains a single BODIPY® dye per vancomycin molecule.

All three dyes were suitable to determine the absolute cell count in *S. aureus* cultures. Additionally, Vancomycin BODPY FL conjugate was suitable for staining internalized bacteria from cell lysate since bacterial and host derived populations could be clearly distinguished. Thus, we provide a fast and exact counting method which could probably be applied to other bacteria in pure cultures or even infection settings when Gram-positive pathogens are employed.
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We hope you had a great DGfZ 2014 meeting!

Bon Voyage...

....and looking forward to seeing you next year at the

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