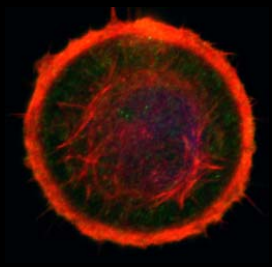
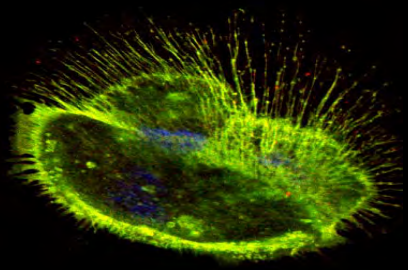
23rd Annual Conference of the German Society for Cytometry

October 9th-11th 2013
Center for Regenerative Therapies, **Dresden**

DGfZ 2013



CYTOMETRY ACROSS DISCIPLINES



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Dear Participants and Guests:

Welcome to the 23rd Annual Conference of the DGfZ e.V. We hope you will find the Dresden environment and the Center for Regenerative Therapies a wonderful and inspiring site to meet with colleagues and share ideas on the topic of this year's meeting "Cytometry Across Disciplines"

Enjoy the multidisciplinary program which bridges the gap between multiple cytometrical and cell analytical methodologies and covering an interesting range of hot topics in biomedical and environmental research.

Along with the scientific sessions, join us for conversation and relax at the Welcome Reception on Wednesday evening and a surprising Conference Dinner on Thursday night.

We also encourage you to visit the Industrial Exhibition. The meeting would not have been possible without the great contribution of sponsors and exhibitors!

Let us know if we can be of any assistance. The registration desk is open for you throughout the entire meeting.

Wishing you an exciting scientific meeting!

Your local organizers

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

Denis Corbeil

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Leoni A. Kunz-Schughart

Denis Corbeil

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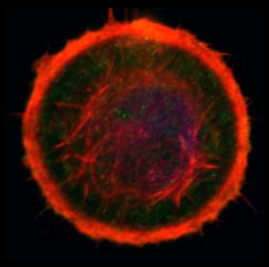
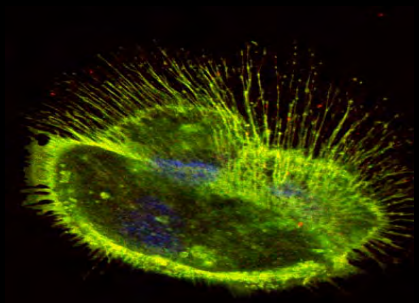


PROGRAM



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Sessions

Social Events

Poster Sessions & Breaks

DGfZ Members

OVERVIEW

Wednesday - 09/Oct/2013	
12:30 - 12:40 pm	**WELCOME**
12:40 - 02:00 pm	**Session 1: Tools for Stem Cell Research**
02:00 - 03:15 pm	**Session 2: Imaging Cell and Tissue Niches**
03:15 - 03:45 pm	**Coffee Break**
03:45 - 05:15 pm	**Session 3: Tracking Cells *in vivo***
05:15 - 07:00 pm	**Welcome Reception**
07:00 - 10:00 pm	**Session 4: Core Manager Workshop**
Thursday - 10/Oct/2013	
08:30 - 09:45 am	**Session 5: Biomarker Signatures**
09:45 - 10:10 am	**Coffee Break**
10:10 - 11:40 am	**Session 6: Emerging Technologies**
11:40 - 12:25 am	**Poster Session I**
12:00 - 01:00 pm	**Lunch**
12:30 - 01:15 pm	**Poster Session II**
01:20 - 03:00 pm	**Session 7: Monitoring (Malignant) Disease & Therapy Responses**
03:00 - 03:30 pm	**Coffee Break & Poster Viewing**
03:30 - 04:30 pm	**Session 8: Cutting-Edge Animal Models of Disease**
04:30 - 05:30 pm	**Session 9: Klaus-Goerttler-Session - Microbiology beyond Biomedicine**
05:30 - 05:45 pm	**Coffee Break**
05:45 - 07:15 pm	**Meeting of the DGfZ Members**
08:00 - 12:00 pm	**CONFERENCE DINNER**
Friday - 11/Oct/2013	
09:00 - 10:30 am	**Session 10: Nanobiotechnology/Nanobiomedicine**
10:30 - 11:00 am	**Coffee Break**
11:00 - 12:30 pm	**Session 11: Cytometry meets Bioinformatics**
12:30 - 01:30 pm	**Lunch Break**
01:30 - 02:30 pm	**Session 12: Meet the Expert Lecture**
02:30 - 04:00 pm	**Session 13: Monitoring Immune Responses**
04:00 - 04:30 pm	**FAREWELL & Coffee**

Wednesday - 09/Oct/2013

12:30 - 12:40 pm	**WELCOME - 23rd Annual Conference of the DGfZ "Cytometry Across Disciplines"** **Leoni Kunz-Schughart**, Dresden, Germany
12:40 - 02:00 pm	**Session 1: Tools for Stem Cell Research** Chair: **Björn Scheffler**, Bonn, Germany Chair: **Tara Walker**, Dresden, Germany **Beyond the Boundaries of Cellular Heterogeneity in Glioblastoma** **Björn Scheffler** (Bonn, Germany) **Are Murine Mesenchymal Stromal Cells a Trap for Human Hematopoietic Stem and Progenitor Cells?** **Doreen Reichert** (Dresden, Germany), **Jens Friedrichs, Steffi Ritter, Theresa Merkel, Carsten Werner, Martin Bornhäuser, Denis Corbeil** **Cord Blood Derived Endothelial Colony Forming Cells Emerge from a CD45dim CD31^{+} Circulating Precursor** **Karen Bieback** (Mannheim, Germany), **Susanne Elvers Hornung, Stefanie Uhlig, Harald Klüter** **Novel Markers for the Prospective Isolation of Neural Stem Cells from the Adult Murine Hippocampus** **Tara Walker** (Dresden, Germany), **Klaus Fabel, Steffen Vogler, Daniela Lasse, Ann Wierick, Alex M Sykes[3], Denis Corbeil[4], Gerd Kempermann**
02:00 - 03:15 pm	**Session 2: Imaging Cell and Tissue Niches** Chair: **Denis Corbeil**, Dresden, Germany Chair: **Ingo Roeder**, Dresden, Germany **CD133 Reveals New Insights into the Cell Biology of Stem and Progenitor Cells** **Denis Corbeil** (Dresden, Germany), **Michaela Wilsch-Bräuninger, Jana Karbanová, Wieland B. Huttner, Martin Bornhauser, Doreen Reichert, Nicola Bauer** **Intravital Imaging of Tumor Cells and Their Microenvironment** **Rinske Drost** (Utrecht, Niederlande), **Saskia Ellenbroek** ***In Vivo* Time Lapse Imaging of Mouse Bone Marrow Reveals Differential Niche Engagement** **Narges Rashidi** (London, UK)
03:15 - 03:45 pm	**Coffee Break**

03:45 - 05:15 pm	**Session 3: Tracking Cells *in vivo*** Chair: **Anja Hauser**, Berlin, Germany Chair: **Ernst Stelzer**, Frankfurt, Germany **High-resolution Intravital Imaging using Striped-Illumination 2-Photon Microscopy** **Zoltan Cseresnyes** (Berlin, Germany), **Laura Oehme, Robert Günther, Uta Höpken, Kristina Schradi, Matthias Richter, Anje Sporbert, Anja Hauser, Raluca Niesner** **Novel Peptide-Based Targeting Probe for *in vivo* Imaging of Membrane Hsp70 Positive Tumors and Metastases** **Stefan Stangl** (Munich, Germany), **Julia Varga, Marija Trajikowich-Arsich, Jens Siveke, Florian Greten, Vasilis Ntziachristos, Gabriele Multhoff** **High Resolution Three-Dimensional Imaging of Cellular Spheroids with Light Sheet-Based Fluorescence Microscopy** **Ernst Stelzer** (Frankfurt, Germany), **Christian Mattheyer, Francesco Pampaloni** **Dynamics of Memory CD8 T Cell Responses** **Wolfgang Kastenmüller** (Bonn, Germany)
05:15 - 07:00 pm	**WELCOME RECEPTION**
07:00 - 10:00 pm	**Session 4: Core Manager Workshop** Chair: **Elmar Endl**, Bonn, Germany **Core Technologies for Life Sciences: Robust Core Services Infrastructure Support Technology Development, Innovation and Discovery** **Spencer L. Shorte** (Paris, France)

Thursday - 10/Oct/2013

08:30 - 09:45 am	**Session 5: Biomarker Signatures** Chair: **Anna Dubrovska**, Dresden, Germany Chair: **Shoutian Zhu**, La Jolla, CA, USA **ALDH1 as a Marker of Radioresistance Within the Prostate Cancer Progenitor Population** **Anna Dubrovska** (Dresden, Germany) **Selectively Inducing Leukemia Stem Cell Differentiation Using Small Molecule Inhibitor of Myc Transcription** **Shoutian Zhu** (La Jolla, CA, USA) **Neuropilin 2 (NRP2) Mediated CXCL12/CXCR4 Signaling in the Formation of Lymph Node Metastasis of Colon Cancer** **H. Schneider** (Dresden, Germany), **P. Hönscheid, R. P. Singh, B. Wielockx, G.B. Baretton, K. Datta, M. Muders**
09:45 - 10:10 am	**Coffee Break**
10:10 - 11:40 am	**Session 6: Emerging Technologies** Chair: **Wolfgang Beisker**, München, Germany Chair: **Antje Dietrich**, Dresden, Germany **Fast Detection of Protein-Protein Interactions with an Automated FRET-based System on the Flow Cytometer** **Kerstin von Kolontaj, Martin Büscher, Gabor Horvath** **The Celigo®: Imaging Cytomety** **Scott Charles Cribbes** **Chromocyte: An Online Resource for the Flow Cytometry Community** **Alan Graham Pockley** **Kaluza for Gallios: A New Acquisition Software** **Michael Braun** **UNCLE DOC - Automatic Analysis of Multi-Parameter Flow Cytometric Data Using an UNsupervised CLustering Engine** **Martin Büscher** ***In Vivo* Multicolor Cellular Imaging for Translational Research** **Hedi Gharbi**
11:40 - 12:25 am	**Poster Session I**
	Lunch
12:30 - 01:15 pm	**Poster Session II**

01:20 - 03:00 pm	**Session 7: Monitoring (Malignant) Disease & Therapy Responses** Chair: **Thomas Kroneis**, Graz, Austria Chair: **Gabriele Multhoff**, Munich, Germany **Circulating Tumor Cells: Current State and Future Perspectives** **Sabine Riethdorf** (Hamburg, Germany)**, Klaus Pantel** **Diagnostic Profiling of Single Circulating Tumor Cells** **Bernhard Polzer** (Regensburg, Germany) **Dissecting Cell Cycle Control and Proliferation on the Cellular Level – Lessons from the Survivin-RNAi-Phenotype** **Hanns Achim Temme** (Dresden, Germany)**, Ralf Wiedemuth, Gabriele Schackert** **Monitoring of Minimal Residual Disease (MRD) in Malignant Hematologic Diseases by Flow Cytometry** **Uta Oelschlägel** (Dresden, Germany)
03:00 - 03:30 pm	**Coffee Break & Poster Viewing**
03:30 - 04:30 pm	**Session 8: Cutting-Edge Animal Models of Disease** Chair: **Anja Wege**, Regensburg, Germany Chair: **Georg Breier**, Dresden, Germany **Zebrafish Xenografts as a Tool for *in vivo* Studies on Human Cancer** **Martina Konantz, Claudia Lengerke** (Tuebingen, Germany) **Humanized Tumor Mice - New Concepts for Translational Research** **Anja Wege** (Regensburg, Germany)**, Katharina Schardt, Olaf Ortmann, Ernst Michael Jung, Alexander Krömer, Gero Brockhoff**
04:30 - 05:30 pm	**Session 9: Klaus-Goerttler-Session - Microbiology beyond Biomedicine** Chair: **Susanne Günther**, Leipzig, Germany Chair: **Lars M. Blank**, Aachen, Germany **Industrial Biotechnology - Can Cytometry Help to Meet the Challenges?** **Thomas Bley** (Dresden, Germany) **Flow Cytometry of Archaea** **Rolf Bernander** (Stockholm, Sweden) **Antigen-Reactive T Cell Enrichment for Direct, High Resolution Analysis of the Human Naïve, Memory and Regulatory $CD4^+$ T Cell Repertoire** **Petra Bacher** (Berlin, Dresden)**, Anne Schönnbrunn, Janka Teutschbein, Olaf Kniemeyer, Mario Assenmacher, Axel A. Brakhage, Andreas Thiel, Alexander Scheffold**
05:30 - 05:45 pm	**Coffee Break**
05:45 - 07:15 pm	**Meeting of the DGfZ Members**
08:00 - 12:00 pm	**CONFERENCE DINNER**

Friday - 11/Oct/2013

09:00 - 10:30 am	**Session 10: Nanobiotechnology/Nanobiomedicine** Chair: **Wolfgang Fritzsche**, Jena, Germany Chair: **Ulrike Taylor**, Mariensee, Germany **Conjugated Gold Nanoparticles as Selective Imaging Tools in Cell Biology** **Stephan Barcikowski** (Duisburg, Germany) **Toxic Effects of Silver Nanoparticles on Neural Cells: Uptake, Oxidative Stress and Acute Calcium Responses in Primary Mixed Neural Cell Cultures** **Georg Reiser** (Magdeburg, Germany) **Nanoplasmonics in Biomedicine: From Thermodynamics at the Nanoscale to Remote Release from Polymeric Capsules, Lipid Systems, Red Blood Cells** **Andre Skirtach** (Potsdam, Germany) **Labeling of *Staphylococcus aureus* with Fluorescent or Para-magnetic Nano-particles Highlights new Capabilities for Following Host-Pathogen Interactions** **Kristin Surmann, Maren Depke, Petra Hildebrandt** (Greifswald, Germany), **Nico Jehmlich, Stephan Michalik, Sarmiza E. Stanca, Wolfgang Fritzsche, Uwe Völker, Frank Schmidt**
10:30 - 11:00 am	**Coffee Break**
11:00 - 12:30 pm	**Session 11: Cytometry meets Bioinformatics** Chair: **Susann Müller**, Leipzig, Germany Chair: **Frank Schmidt**, Greifswald, Germany **Image-Based Quantification of Cellular Dynamics** **Ingo Roeder** (Dresden, Germany) **OmicsData and Visualization – What's in the haystack?** **Jörg Bernhardt** (Greifswald, Germany) **Revealing Regulatory Principles of Functional Heterogeneity in Haematopoietic Progenitor Cells: a Combined Experimental and Modelling Approach** **Enrica Bach, Thomas Zerjatke, Manuela Herklotz, Nico Scherf, Dietger Niederwieser, Ingo Roeder, Tilo Pompe, Michael Cross, Ingmar Glauche** (Dresden, Germany) **A New Approach for Semiautomated Analysis of Multispectral Flow Cytometric Data** **Kristen Feher** (Berlin, Germany), **Jenny Kirsch, Andreas Radbruch, Hyun-Dong Chang, Toralf Kaiser**
12:30 - 01:30 pm	**Lunch Break**
01:30 - 02:30 pm	**Session 12: Meet the Expert Lecture** Chair: **Leoni Kunz-Schughart**, Dresden, Germany **The Future of Cord Blood Stem Cell Banking: Regenerative Medicine & Cellular Therapies** **David Harris** (Tucson, AZ, USA)

02:30 - 04:00 pm	**Session 13: Monitoring Immune Responses** Chair: **Hyun-Dong Chang**, Berlin, Germany Chair: **Karsten Kretschmer**, Dresden, Germany **Single Cell Raman Micro-Spectroscopy – a Powerful Tool in Clinical Diagnosis** **Jürgen Popp** (Jena, Germany) **Reference Values of Leukocyte Subpopulation Counts of Men and Women aged 18-87 Years. First Results from the Analysis of healthy Adults in the Leipzig LIFE study.** **Susanne Melzer** (Leipzig, Germany), **Jozsef Bocsi, Silke Zachariae, Christoph Engel, Markus Löffler, Attila Tárnok** **Counting Cytokine-Producing T Helper Cells: Concentration of Calcium in the Medium Can be Critical** **Jakob Zimmermann** (Berlin, Germany), **Andreas Radbruch, Hyun-Dong Chang** **Modulation of T Lymphocyte Calcium Influx Patterns via the Inhibition of Kv1.3 and IKCa1 Potassium Channels in Autoimmune Disorders** **Gergely Toldi** (Budapest, Hungary) **Fluorochrome-Based Definition of Foxp3$^+$ Regulatory T Cell Subphenotypes with Specialized Suppressor Functions** **Karsten Kretschmer** (Dresden, Germany)
04:00 - 04:30 pm	**FAREWELL COFFEE**

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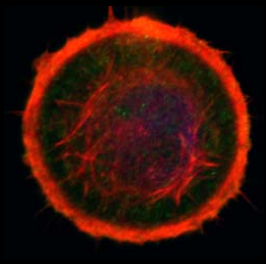
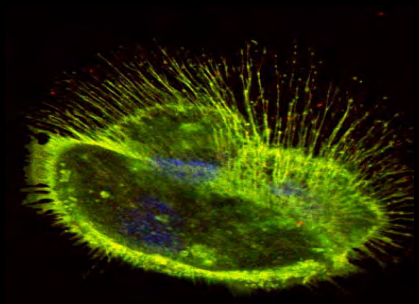


SESSION 1

(Wednesday - 09/Oct/2013, 12:40 - 02:00 pm)

Tools for Stem Cell Research



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Chairs:

Björn Scheffler, University of Bonn, Germany
Tara Walker, CRTD, TU Dresden, Germany

This session discusses the analysis and the monitoring of stem and progenitor cells in health and disease. Particular emphasis will be given to the broad spectrum of tools currently applied to identify, isolate, characterize, and to modulate cellular phenotypes *in vitro* and *in vivo*. The plenary talks will highlight some recent developments and applications of cytometric technologies in the fields of brain development and recovery. Additional presentations will focus on bone marrow and other organ-specific stem and progenitor cell phenotypes, as well as on the identification and the use of respective surrogate markers for technical applications.

Beyond the Boundaries of Cellular Heterogeneity in Glioblastoma

Björn Scheffler
Institute of Reconstructive Neurobiology, University of Bonn, Germany

Cellular heterogeneity is a characteristic finding in high-grade tumors, e.g. the brain tumor glioblastoma that goes along with a poor clinical outcome of affected patients. In the last years, research has begun to recognize that tumor cell diversity is not simply an epiphenomenon of malignant diseases, rather an essential root of therapeutic resistance. This talk will introduce current concepts on the origins, maintenance, and the consequences of tumor cell heterogeneity in human glioblastoma. Based on own experimental evidence, examples for diagnostic and therapeutic implications will be presented.

Are Murine Mesenchymal Stromal Cells a Trap for Human Hematopoietic Stem and Progenitor Cells?

Doreen Reichert[1], Jens Friedrichs[2], Steffi Ritter[1], Theresa Merkel[1], Carsten Werner[2], Martin Bornhäuser[3], Denis Corbeil[1]
[1]Tissue Engineering, BIOTEC; [2]Leibnitz Institute of Polymer Research, Institute of Biofunctional Polymer Materials; [3]Medical Clinic and Polyclinic I, University Hospital Carl Gustav Carus Dresden, Germany

OBJECTIVE Humanized mice give the opportunity to study human biology with less limitation. Many models were developed for the exploration of the human hematopoietic system particularly the stem cell niche. Understanding all molecular details underlying differences between human and mouse system is urged to establish an ideal model.

METHODS To dissect these issues, we compared the proliferative and biochemical properties of human $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) growing on murine multipotent mesenchymal stromal cells (MSCs) by comparison to human ones. In both species, MSCs were isolated from bone marrow, and presence/absence of characteristic cell-surface antigens was determined by flow cytometry. $CD34^+$ HSPCs were immuno-isolated from human peripheral blood upon G-CSF mobilization. After 7 days of co-culture, the phenotype of HSPCs was also determined by flow cytometry and their morphology was observed by scanning electron and time-lapse video microscopies. The adhesion strength between HSPCs and MSCs was evaluated by atomic force microscopy (AFM)-based single-cell force spectroscopy.

RESULTS We observed that human HSPC pools are expanding better on human and murine MSCs by comparison to fibronectin used as supporting matrix. Although the number of $CD34^+CD133^-$ HSPCs increased in similar proportion, those harboring the more primitive $CD133^+$ phenotypes were significantly reduced on murine MSCs indicating a substantial difference on the HSPC-supportive capacity between species. Furthermore, the number of human HSPCs exhibiting morphologies of migration was significantly decreased upon culture on murine MSCs by comparison to human ones. Such functional and morphological differences may be a consequence of distinct adhesive properties of human HSPCs on feeder cell layers originating from human or murine system. Remarkably, we found by means of AFM that detachment forces of human HSPCs are threefold higher on murine MSCs by comparison to human counterparts.

CONCLUSIONS Collectively, our data demonstrate for the first time that the adhesion properties of human $CD34^+$ HSPCs on murine versus human MSCs differ significantly. Such information raises some caution in the interpretation of data obtained with murine models used to study the primitive properties of human HSPCs and their migration behaviour such as the homing process.

Cord Blood Derived Endothelial Colony Forming Cells Emerge from a CD45dim CD31$^+$ Circulating Precursor

Karen Bieback, Susanne Elvers Hornung, Stefanie Uhlig, Harald Klüter
Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, Heidelberg University; German Red Cross Blood Service of Baden-Württemberg – Hessen, Germany

Within the last decade numerous studies have been published dealing with endothelial progenitor cells (EPC). Concomitant it became apparent that a variety of different cell types have been subsumed under the term EPC. Depending on the protocol culture adapted EPC have now been classified as i) colony forming unit endothelial cells (CFU-EC), ii) early outgrowth/ proangiogenic (CAC) or iii) late outgrowth/endothelial colony forming cells (ECFC). For circulating EPC the precise phenotypic definition is still heavily discussed. Different authors propose different combinations of the markers CD34, CD133, VEGFR-2/KDR, CD31 and CD45 debating whether EPC are of hematopoietic origin.

To gain insight into the early phases of the differentiation cascade, the phenotypes of uncultivated CD34$^+$ mononuclear cells (CD34$^+$ MNC), ECFC and HUVEC at primary passage (p0) and p1 were analyzed. Already within p0 ECFC underwent a rapid maturation from a CD45$^+$ and CD31$^+$ phenotype to a CD45 negative, and endothelial marker positive phenotype as defined by flow cytometry and multiphoton imaging. In primary passage, ECFC colonies of homogenous cobblestone morphology contained subpopulations expressing a mature endothelial phenotype, but also subpopulations co-expressing CD45dim and CD31, but no other hematopoietic marker such as CD14, CD41 or CD62p, suggesting that ECFC emerge from a CD45dim/CD31$^+$ precursor. Imaging revealed that CD45 was dimly expressed at the cell surface - only in ECFC but not in HUVEC. Interestingly, few ECFC showed perinuclear CD45 aggregation or intracellular CD45 caps. Compared to HUVEC, ECFC showed a less concise expression of CD31 at the cell-cell-contact sites. Finally our data confirm ECFC as an unique cell population exerting high angiogenic and vasculogenic capacity

Our study supports the notion that ECFC emerge from a CD45dim CD31$^+$ progenitor and very rapidly mature in culture. The data strengthen the necessity to identify a set of markers capable of prospectively discriminating endothelial from hematopoietic cells as well as progenitor from mature cells.

Novel Markers for the Prospective Isolation of Neural Stem Cells from the Adult Murine Hippocampus

Tara Walker[1], Klaus Fabel[1,2], Steffen Vogler[1], Daniela Lasse[1], Ann Wierick[1], Alex M Sykes[3], Denis Corbeil[4], Gerd Kempermann[1,2]

[1]CRTD – Center for Regenerative Therapies Dresden, TU Dresden;
[2]German Center for Neurodegenerative Diseases (DZNE);
[3]Max Planck Institute for Molecular Cell Biology and Genetics;
[4]Tissue Engineering Laboratories (BIOTEC), TU Dresden, Dresden, Germany

Neurogenesis in the adult mouse hippocampus is well characterized, however despite considerable effort there was a distinct lack of appropriate markers for the prospective isolation of the resident stem cells. In our search we have identified Prominin-1 and the lysophosphatidic acid receptor (LPA1) as novel hippocampal precursor cell markers. Prominin-1 (CD133) is commonly used to isolate stem and progenitor cells from the developing and adult nervous system and to identify cancer stem cells in brain tumors. However, no information about the expression of Prominin-1 by precursor cells of the adult hippocampus was available. Using a fluorescence activated cell sorting (FACS) approach we show that a small subset of Prominin-1$^+$ cells co-express the non-specific precursor cell marker Nestin as well as GFAP and Sox2. In addition, following isolation only Prominin-1/Nestin double-positive cells fulfilled the defining stem cell criteria of proliferation, self-renewal and multipotentiality as assessed by a neurosphere assay.

Lysophosphatidic acid (LPA) is an extracellular signaling lysophospholipid that binds to the G protein-coupled receptors LPA1-6 to regulate many important biological processes including neural cell development. FACS followed by neurosphere assays confirmed that the precursor activity was confined to the LPA1-GFP$^+$ cell population with >99% of total neurospheres being formed from the LPA1-GFP$^+$ cells. Surprisingly, the more commonly used precursor cell reporter line Nestin-GFP was a less specific marker of these cells with only 80% of the total neurosphere formation from the Nestin$^+$ population. In addition, LPA1-GFP can be used as a reliable marker of those cells in the dentate gyrus capable of being activated by physical activity, with a significant increase in the number of LPA1-GFP$^+$ cells following 10 days of voluntary running. Finally, by combining LPA1-GFP expression with two cell surface markers, Prominin-1 and EGF-receptor, the purity of the isolated precursor population could be increased from 1/50 to 1/3 cells capable of forming a neurosphere.

In summary, we show that the novel markers Prominin-1 and LPA1-GFP can be used to enrich precursor cells from the adult murine dentate gyrus. In addition, we describe novel marker combinations for the simultaneous flow cytometric isolation of multiple cell types from the adult dentate gyrus. This approach will facilitate the isolation and characterization of homogeneous hippocampal cell populations in order to gain a deeper understanding of the underlying molecular regulation of hippocampal neurogenesis in response to activity or injury in individual cell types rather than at the level of the entire dentate gyrus.

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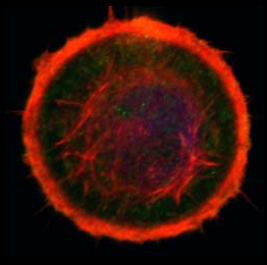
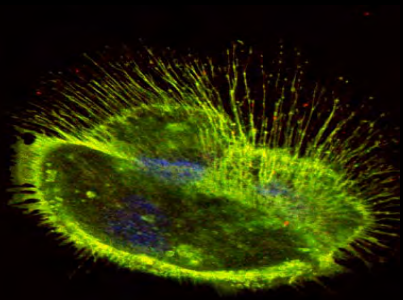


SESSION 2

(Wednesday - 09/Oct/2013, 02:00 - 03:15 pm)

Imaging Cell and Tissue Niches



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Chairs:

Denis Corbeil, Biotec, TU Dresden, Germany
Ingo Roeder, IMB, TU Dresden, Germany

Nowadays, cells with primitive properties such as stem and cancer stem cells are described in the literature as the good and the bad players, respectively. The former are crucial cellular constituents in the development of an organism, and the maintenance of its tissues throughout the adulthood. The latter seem to cause the initiation and progression of cancer. Clinically, stem and cancer stem cells are also considered as putative targets either for regenerative therapy or a therapeutic avenue in cancer therapy. To better dissect the cell biology of these cells in their "natural microenvironment", the so-called stem cell niche, novel *in vivo* imaging methodologies and *ex vivo* culture systems are emerging. The present session will provide an exciting overview on these new tools, which have revealed novel unexpected biological facets of stem and cancer stem cells.

CD133 Reveals New Insights into the Cell Biology of Stem and Progenitor Cells

Denis Corbeil[1], Michaela Wilsch-Bräuninger[2], Jana Karbanová[1], Wieland B. Huttner[2], Martin Bornhauser[3], Doreen Reichert[1], Nicola Bauer[1]
[1]Tissue Engineering Laboratories, BIOTEC, TU Dresden; [2]Max-Planck-Institute of Molecular Cell Biology and Genetics; [3]Medical Clinic and Polyclinic I, University Hospital Carl Gustav Carus, Dresden, Germany

CD133 (alias prominin-1) is a pentaspan membrane glycoprotein concentrated in various types of plasma membrane protrusions (e.g., microvillus, primary cilium, uropod). This cholesterol-binding protein is associated with membrane microdomains (lipid rafts), and several types of stem cells including those found in the neural and hematopoietic system express it. CD133 is widely used as a cell surface marker of cancer stem cells. Previously, we demonstrated that in the developing embryonic mouse brain, CD133 is released from neuroepithelial progenitors into the lumen of the neural tube, concomitant with their differentiation, by means of plasma membrane-derived vesicles referred to as ectosomes. These CD133-positive membrane vesicles are budding from the tips of microvilli and primary cilia. Clinically, their level is up regulated in cerebrospinal fluid of glioblastoma patients. Do similar phenomena exist in other stem cell types or is it unique to those derived from the neural system? Is the release of CD133-positive membrane vesicles a read out of differentiation in general? We have investigated these questions using human CD133-positive hematopoietic stem and progenitor cells (HSPCs) growing on primary multipotent mesenchymal stromal cells (MSCs) as a feeder cell layer. We report here the following observations. First, CD133 is released from HSPCs into the culture medium in association with membrane vesicles that are sedimented after high-speed centrifugation. Second, these CD133-positive vesicles are enriched in membrane cholesterol, and contain Flotillin-1/2 and Syntenin-1 – a PDZ domain containing protein that interacts with the exosomal marker CD63 – raising the possibility that these vesicles originate not only from the plasma membrane protrusions, but also from intracellular structures. Third, the differential immunofluorescence revealed that CD133, in addition to its association with plasma membrane protrusions, is present in intracellular compartments, which at the electron microscopy level appear as multivesicular bodies demonstrating the association of CD133 with exosomes. Fourth, the amount of CD133-positive membrane vesicles found in the culture medium increases upon cultivation whereas the number of CD133-positive cells is decreasing, indicating a general link between the release of CD133-positive membrane vesicles and cellular differentiation.

Intravital Imaging of Tumor Cells and Their Microenvironment

Rinske Drost, Saskia Ellenbroek
Hubrecht Institute, Utrecht, The Netherlands

Complications due to metastasis, the process in which cells detach from a primary tumor to form new tumors at distant sites, are the primary cause of cancer-related death. Although histological techniques have provided important information on metastasis, they only give a static image of tumor cells and their microenvironment and thus compromise the interpretation of this dynamic process. To study this dynamic process, we visualize the behavior of single metastasizing cells at subcellular resolution with two-photon intravital imaging (IVM). We have developed a Mammary Imaging Window (MIW) to image primary mammary tumors over multiple days. In addition, we have developed an Abdominal Imaging Window (AIW) to study the processes involved in metastasis. Together with intravital lineage tracing tools we are able to visualize individual tumor cells and their progeny in the same animals in primary tumors and metastases over periods of weeks. We monitor individual tumor cells and their progeny over periods of weeks in primary tumors and metastases. We show the existence of cancer stem cells (CSC) in genetic mammary tumors and illustrate the dynamic nature of these cells by visualizing the disappearance and formation of CSCs during the growth and progression of a tumor. Moreover, in the same genetic mouse model, we show that tumor cells share lipids, proteins and RNA, locally and systemically. Intravitally we show that this local and systemic transfer of biomolecules by tumor cell-released microvesicles is a physiological process that is directly coupled to the migratory behavior of recipient breast cancer cells. Lastly, we visualize how individual tumor cells arrive, survive and colonize metastasis-prone sites such as the liver. Surprisingly, we identified that cell migration contributes to metastatic growth even after the initiation of metastases. From all our studies we conclude that the dynamic behavior of a small population of tumor cells is responsible for the spread and growth of tumors.

In Vivo Time Lapse Imaging of Mouse Bone Marrow Reveals Differential Niche Engagement

Narges Rashidi
Division of Cell & Molecular Biology, Imperial College London, London, United Kingdom

Understanding the mechanisms linking stem cell-niche interaction and stem cell fate is critical for developing regenerative medicine approaches. The nature of such interactions between hematopoietic stem cells (HSC) and the bone marrow (BM) microenvironment has long been elusive due to the difficulty of penetrating bones for direct observation and the fluid nature of the hematopoietic tissue itself. Several functional studies based on ablating or overexpressing specific genes in the hematopoietic or distinct BM stroma compartments have highlighted the presence of an intricate and dynamic network of regulatory signals responsible for the crosstalk between HSC and the BM microenvironment. The question, however, remains open as to whether multiple, molecularly and functionally distinct HSC niches exist within the bone marrow and whether HSC trafficking between them may be necessary to switch fate between quiescence and proliferation, self-renewal and differentiation.

To address this question, we developed an imaging technique combining two photon and confocal microscopy that allows *in-vivo* imaging of live transplanted hematopoietic stem and progenitor cells (HSPC) in mouse BM with single cell resolution. Using this technique we showed that engrafting long-term repopulating HSC (LT-HSC) localize near osteoblastic cells, while their progeny are more distal. Our results also highlight that localization of LTHSC and their progeny near osteoblasts correlates with improved engraftment outcomes. Studies based on single time-point observations demonstrated that asynchronous HSPC proliferation initiates BM reconstitution, however did not provide information about long-term interactions between HSC and their BM niche (or niches), which are responsible for maintenance of balanced hematopoiesis. We therefore developed a new *in vivo* imaging experimental protocol, allowing time-lapse imaging of HSPC, leading us to uncover their differential abilities to engage with the BM microenvironment over time. Moreover, using a physiological model of HSC activation, we observed that changes in the nature of the interactions between stem cells and the BM microenvironment accompany switches in fate choice.

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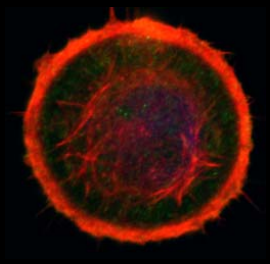
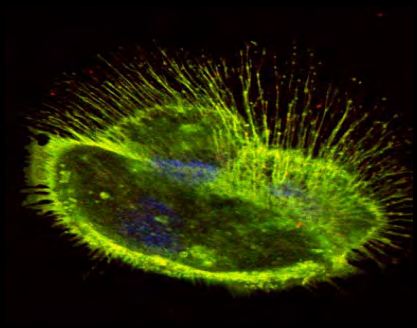


SESSION 3

(Wednesday - 09/Oct/2013, 03:45 - 05:15 pm)

Tracking Cells *in vivo*



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Chairs:

Anja Hauser, DRFZ, Berlin, Germany
Ernst Stelzer, Goethe University, Frankfurt, Germany

The visualization of large, living samples at sub-cellular resolution over extended time periods has become the method of choice in order to understand biological processes within their spatiotemporal context. It is therefore becoming increasingly important in several areas of life science, for example in immunology, neuro-science or developmental biology. In the "Tracking Cells *in vivo*" session, recent developments in the field of intravital imaging techniques are discussed. In particular, two-photon imaging and light sheet-based fluorescence microscopy will be the focus of this session. Besides recent technical advancements, an emphasis will be put on the applications as well as on innovative ways of analyzing the complex data sets generated with these techniques.

High-resolution Intravital Imaging using Striped-Illumination 2-Photon Microscopy

Zoltan Cseresnyes[1], Laura Oehme[1], Robert Günther[1], Uta Höpken[2], Kristina Schradi[2], Matthias Richter[2], Anje Sporbert[2], Anja Hauser[1], Raluca Niesner[1]
[1]DRFZ, Germany; [2]MDC-Berlin, Germany

Intravital microscopy presents multiple challenges for scientist: there is a depth-dependent decrease of the signal-to-noise ratio (STNR) of the acquired images, there exists a significant loss of the optical resolution, and it is difficult to analyze such images quantitatively due to the light-scattering induced optical aberrations. The spatial resolution loss is especially apparent in the axial direction, which is particularly harmful because the diffraction-limited theoretical optical resolution is already 3-times worse in the axial direction than in the lateral one. This emphasizes the importance of finding novel ways to improve the Z resolution during deep-tissue multi-photon laser scanning microscopy (MPLSM).

Here we present a new method, called Striped-Illumination MPLSM (SI-MPLSM). Unlike structured-illumination methods, SI-MPLSM is not based on wide-field technology, but rather provides a laser scanning, camera-based imaging method. Here we apply the LaVision/Biotec TriMScope's multi-beam EM-CCD-based operating method, and we use the 16- or 32-beam periodic illumination pattern to generate a series of images at equidistantly shifted positions performed perpendicularly to the scanning direction. From these images we calculate the increased resolution and higher contrast final image using custom-made algorithms. We will present the effect of the various algorithms on the final outcome, as well as the deeper details of the calculations. The optical resolution values achieved by traditional methods (PMT or CCD-camera based) and by SI-MPLSM will also be presented and compared.

Novel Peptide-Based Targeting Probe for *in vivo* Imaging of Membrane Hsp70 Positive Tumors and Metastases

Stefan Stangl[1], Julia Varga[2], Marija Trajikowich-Arsich[3], Jens Siveke[3], Florian Greten[2], Vasilis Ntziachristos[4], Gabriele Multhoff[1]

[1]Klinikum rechts der Isar, Dpt. Radiation Oncology, TU München and Helmholtz Zentrum München (HMGU); [2]Klinikum rechts der Isar, Dpt. Gastroenterological Oncology; [3]Klinikum rechts der Isar, Medical Faculty II; [4]HMGU, Institute of Biological and Medical Imaging, Munich, Germany

The major stress-inducible Heat shock protein 70 (Hsp70, Hsp1A1) is frequently overexpressed in highly aggressive human, as well as murine tumors, since elevated intracellular Hsp70 levels mediate protection against apoptosis. Hsp70 plays a key role in development, viability and metastasizing capacities of tumor cells *in vivo*.

The binding capacities of a protein-targeted contrast agent for the imaging of Hsp70 membrane-positive tumors was tested *in vitro* and *in vivo*. In contrast to normal tissues, membrane-bound Hsp70 is found on a wide variety of primary tumors, as well as metastases. Here, we report on a novel peptide-based targeting probe addressing membrane-bound Heat shock protein 70 (Hsp70) on tumors and metastases in syngeneic, xenograft and spontaneous mouse models. The probe recognizes the oligomerization domain of Hsp70 that is presented on the cell surface of a broad variety of primary tumors (human and mouse) and metastases, but not on normal tissues. Due to the rapid turnover of membrane Hsp70 at 37°C the fluorescence-labeled targeting probe specifically internalizes in tumor cells. After intravenous (i.v.) injection into the tail vein, the Hsp70 probe, but not a scrambled control peptide, enables a site-specific labelling of tumors and metastases in different tumor mouse models with a minimum size of 0.1 mm in diameter. Biodistribution of the Hsp70 probe revealed a maximum enrichment of the fluorescence signals within the tumor 24 h after i.v. injection. A comparison of our probe with the commercial available imaging probe integrisense revealed highly better tumor to background ratios by using the Hsp70 probe.

We expect that multimodal imaging of membrane Hsp70 positive tumors and metastases using Hsp70 probe might be useful for clinical diagnosis, as well as therapeutic monitoring of tumors and metastases in the future, and might help to accelerate the development of Hsp70-based targeting drugs.

High Resolution Three-Dimensional Imaging of Cellular Spheroids with Light Sheet-Based Fluorescence Microscopy

Ernst Stelzer, Christian Mattheyer, Francesco Pampaloni
Physical Biology, Goethe University Frankfurt, Germany

Conventional two-dimensional cell monolayers do not provide the geometrical, biochemical, and mechanical cues found in tissues. In fact, the cells that form the tissues interact through chemical and mechanical stimuli with adjacent cells and via the extracellular matrix (ECM). Such a highly interconnected communication network extends along all three dimensions. This architecture is lost in two-dimensional cultures. Therefore, two-dimensional cell monolayers do not represent a suitable *in vitro* tool to accurately characterize the biology of most tissues. Many studies performed over the last years have demonstrated that the differences between three-dimensional and two-dimensional cultured cells are striking at the morphological and at the molecular level, and that three-dimensional cell cultures can be employed in order to shrink the gap between tissues and *in vitro* cell models. End-point and long-term imaging of cellular and sub-cellular processes with fluorescence microscopy provide a direct insight into the physiological behavior of three-dimensional cell cultures and their response to chemical or mechanical stimulations. Fluorescence imaging of three-dimensional cell cultures sets new challenges and imposes specific requirements concerning the choice of a suitable microscopy technique. Deep penetration into the specimen, high imaging speed, and ultra-low intensity of the excitation light are key requirements. Light sheet-based fluorescence microscopy (LSFM) offers a favorable combination of these requirements. Therefore, this microscopy technique is currently established as the technique of choice for the study of three-dimensional cell cultures. The development of LSFM draws on many previous developments. In particular, confocal theta fluorescence microscopy played a very important role. About a dozen papers on theta microscopy describe its properties and that of LSFM (single & two-photon, annular/Bessel beams, a/symmetric arrangements …) theoretically as well as practically.

All references are found via: http://www.researcherid.com/rid/A-7648-2011. The talk illustrates the benefits of cellular spheroids in the modern life sciences and suggests that LSFM is essential when investigating cellular and sub-cellular dynamic processes in three-dimensions over time and space.

Dynamics of Memory CD8 T Cell Responses

Wolfgang Kastenmüller
Institutes of Molecular Medicine and Experimental Immunology (IMMEI), University of Bonn, Germany

After an infection, the immune system generates long-lived memory lymphocytes whose increased frequency and altered state of differentiation enhance host defense against reinfection. Flow cytometry allows highly quantitative analysis of these complex memory populations, yet at the cost of neglecting their tissue localization. However, the spatial distribution of memory cells was found to contribute to their protective function. Effector memory $CD8^{(+)}$ T cells reside in peripheral tissue sites of initial pathogen encounter, in apparent anticipation of reinfection.

In order to analyze memory $CD8^{+}$ T cells dynamics and localization in lymph nodes we describe an analytical microscopy method, "histo-cytometry," for visualizing and quantifying phenotypically complex cell populations directly in tissue sections. Using histo-cytometry and intravital imaging we show that within LNs, memory $CD8^{(+)}$ T cells were concentrated near peripheral entry portals of lymph-borne pathogens, promoting rapid engagement of infected sentinel macrophages. A feed-forward CXCL9-dependent circuit provided additional chemotactic cues that further increase local memory cell density. Memory $CD8^{(+)}$ T cells also produced effector responses to local cytokine triggers, but their dynamic behavior differed from that seen after antigen recognition. These data reveal the distinct localization and dynamic behavior of naive versus memory T cells within LNs and how these differences contribute to host defense.

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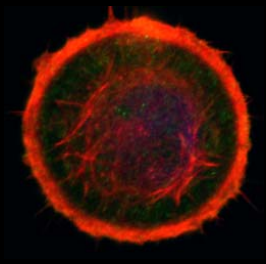
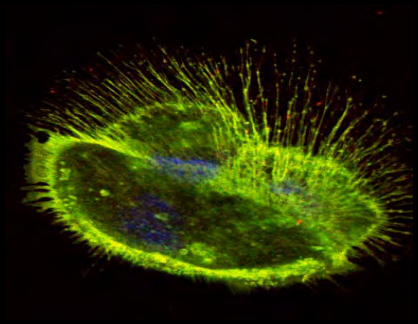


SESSION 4

(Wednesday - 09/Oct/2013, 07:00 - 10:00 pm)

Core Manager Workshop



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Chair:

Elmar Endl, University of Bonn, Germany
Spencer L. Shorte, Institut Pasteur, Imagopole, Paris, France

This workshop is intended to give an overview on new techniques and tools to keep life and work in the Core as friendly and up to date as possible. Technical advancements, facility requirements, management and organisation of users, principal investigators and faculty, knowledge resources and networks, are just a few of the topics to be discussed. But most importantly this workshop should be a venue for people to meet and share their experiences in running a core facility, regardless of whether they just started to build up their own facility or if they are considered to be experts in the field.

Core Technologies for Life Sciences: Robust Core Services Infrastructure Support Technology Development, Innovation and Discovery

Spencer L. Shorte
Institut Pasteur, Imagopole, Paris, France

Life Sciences research seeks to establish experimental approaches using functional analyses of healthy and disease models. Consequently, the systematic routine use of state-of-the-art technologies for multiplexed analyses of functional and molecular dynamics of living systems from molecule, to cell, to tissue, and ultimately *in situ* and *in vivo* is paramount. In this context, new technologies development, and experimental methods define the cutting-edge, wherein innovation can lead to the revelation of unexpected insight. However, technology innovation per se is a risky-business, with high-attrition, that does not sit well within the organization or budget constraints of traditional thematic life science research. To address the deficit, an emergent model being pioneered by life science research institutes around the world uses appropriately organized Core Facility Services (CFS) as an infrastructure platform to harbor technology research and development. The CFS niche provides research scientists and engineers the opportunity to gestate new technology innovation and its adaptation for experimental biology in a dedicated environment, notably outside of the research laboratory. Describing our experiences efforts, successes and failures at the Institut Pasteur Paris this presentation will seek to give insight upon how as a community resource CFS can help better minimize the risk and augment the benefits of concerted technology development. Notably, such that innovative technologies, methods, and experimental paradigms may be optimized that they are better able to deliver results bearing greater relevance to the biology they aim to study.

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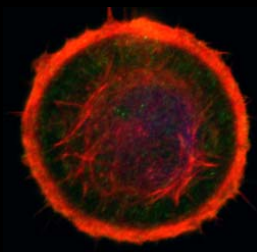
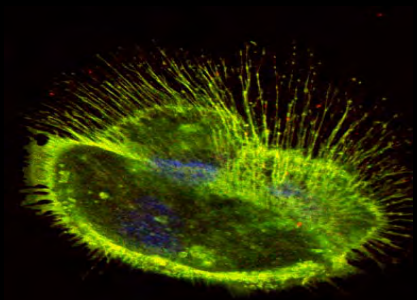


SESSION 5

(Thursday - 10/Oct/2013, 08:30 - 09:45 am)

Biomarker Signatures



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OncoRay National Center for Radiation Research in Oncology Dresden

Chairs:

Anna Dubrovska, OncoRay, TU Dresden, Germany
Shoutian Zhu, California Institute for Biomedical Research, USA

Cytometry methods have the unique potential to analyze and identify complex cell populations and to provide information on the functional status of regulatory processes by simultaneously detecting and quantifying multiple cellular phenotypes and characteristics. These measurable cell parameters - or biomarkers - are used as indicators in health and disease, e.g. for normal biological processes, pathological conditions or therapeutic responses. The diagnosis and management of many types of cancers, for example, have been impacted by the discovery and validation of a wide variety of tumor markers and biomarker signatures. Biomarkers that are deregulated in cancer stem cells are of particular interest because they not only serve as potential targets in cancer therapy but also may be used to predict treatment outcome and to select the optimal therapeutic strategy, and thus may facilitate a personalized approach for cancer treatment. The session will thus primarily but not exclusively focus on new biomarkers in cancer research.

ALDH1 as a Marker of Radioresistance Within the Prostate Cancer Progenitor Population

Anna Dubrovska
OncoRay - National Center for Radiation Research in Oncology, TU Dresden, Germany

A large body of evidence has demonstrated that many human tumors are maintained by a small cell population called cancer stem cells (CSCs) or tumor progenitors, which are responsible for tumor formation, therapy resistance and metastasis. Moreover, this population has been implicated in resistance to chemo- and radiotherapy and tumor recurrence. If challenged with different stimuli coming from the microenvironmental niche and cancer therapies, non-CSCs may undergo a reprogramming, which results in generation of induced CSC (iCSC) cell population. We found that radiation treatment enriches CSC phenotype and properties by preferential survival and expansion of tumor progenitor cells along with reprogramming of non-CSC cell population. Our studies revealed that aldehyde dehydrogenase (ALDH) activity is indicative for prostate tumor progenitor cells with increased chemo- and radioresistance, enhanced migratory potential, improved DNA- double strand break repair and activation of the signaling pathways, which promote therapy resistance, self-renewal and epithelial-mesenchymal transition. Our studies suggest that CSC markers are indicative for radioresistant prostate cancer cell population, and combination of irradiation with therapies directed against CSCs might increase tumor radiocurability.

To develop the radioresistant sublines of the established cancer cell lines, prostate cancer cells were treated with multiple low doses of X-rays (more that 40Gy in total). The radioresistant prostate cancer cells share many properties with tumor progenitor cells including an enhanced expression of CSC markers (CD133, CXCR4, ABCG2, OCT4, NANOG), high aldehyde dehydrogenase (ALDH) activity, activation of epithelial-mesenchymal transition (EMT) and an increase in DNA repair capacity.

Whole genome gene expression profiling of cancer cells, their radioresistant derivatives and tumor progenitor populations revealed common signaling pathways, which link CSCs to radioresistance and include Wnt/β-catenin, PI3K/AKT, G protein-coupled receptor, TGFβ and Integrin signaling pathways.

We showed that radioresistant tumor progenitor population undergoes a phenotypic switching during the course of irradiation, suggesting that controlling the phenotypical and functional properties of CSCs during radiation therapy is important for optimization of treatment strategy.

Our finding has shown that radioresistant properties of cancer progenitor population may be dynamic in nature and can be differently employed throughout the course of radiotherapy. Thereby, different therapeutic strategies and different predictive biomarkers may be required at the different stage of tumor treatment. Our results indicate that therapies that specifically target pathways, which are deregulated in tumor-initiating radioresistant prostate cancer stem cells, such as WNT/β-catenin signaling, may enhance the efficiency of radiotherapy in the future.

Selectively Inducing Leukemia Stem Cell Differentiation Using Small Molecule Inhibitor of Myc Transcription

Shoutian Zhu
California Institute for Biomedical Research, La Jolla, CA, United States of America

Cancer stem cells (CSCs) are attractive target cell populations for novel therapeutic strategies. Many CSCs arise from abnormal stem and progenitor cells and share molecular and phenotypic characteristics with normal stem cells residing in the same tissues. Strategies targeting CSCs, such as forced differentiation and induced cell death, usually do not distinguish between CSCs and their non-cancerous counterparts, thus may exhaust the latter and loss regeneration potential. Identification of mechanisms and development of strategies distinguishing between CSCs and normal stem cells provide avenues for both basic stem cell biology and clinical research.

c-Myc is upregulated in leukemia stem cells (LSCs), whose suppression is associated with LSC differentiation and the loss of tumorigenic potential. In long term hematopoietic stem cells (LT-HSCs), c-Myc level is low, its upregulation is associated with HSC mobilization and differentiation. The difference in the c-Myc levels promises possibilities to distinguish between the two stem cell populations. Using small molecule inhibitor of c-Myc transcription, we are trying to selectively target LSCs for differentiation and spare LT-HSCs, which may eliminate the need of bone marrow transplantation conventionally practiced following radiation or chemotherapy in leukemia patients.

Transcription factors are conventionally considered "not druggable". The application of small molecule inhibitor of c-Myc transcription provides a proof-of-concept for transcription factors and other protein families as therapeutic targets.

Neuropilin 2 (NRP2) Mediated CXCL12/CXCR4 Signaling in the Formation of Lymph Node Metastasis of Colon Cancer

H. Schneider[1], P. Hönscheid[1], R. P. Singh[1], B. Wielockx[1], G.B. Baretton[1], K. Datta[2], M. Muders[1]

[1]Institute of Pathology, University Hospital Carl Gustav Carus, TU Dresden, Dresden, Germany; [2]Department of Molecular Biology and Biochemistry, University of Nebraska Medical School, Omaha, Nebraska, USA

Colon cancer is the third most common cancer in humans. The metastatic dissemination of cancer cells is one of the most important causes of death in cancer patients. We have already shown, that the presence of lymph vessels inside regional lymph nodes is an independent prognostic factor in colorectal cancer (Jakob et al., Plos One, 2011). Therefore, we hypothesize that these lymphatic vessels produce factors that attract tumor cells to the lymph nodes. It has already been shown that CXCL12, also known as SDF1alpha, is produced by lymphatic endothelial cells and binds to its ligand C-X-C motif receptor 4 (CXCR4), which is present on the cell surface of cancer cells. Here, we evaluate the role of Neuropilin-2 (NRP-2) in the regulation of the CXCL12/CXCR4 axis.

In line with a previously published study in breast cancer (Yasuoka et al., BMC Cancer, 2009), transient depletion of NRP2 by siRNA in the colon cancer cell line SW480 leads to a decrease of CXCR4 messenger and protein expression. Furthermore, recombinant human CXCL12 triggered the messenger expression of NRP2 in SW480 cells, while CXCR4 itself does not influence the expression of NRP2. Blocking NRP2 abrogates CXCL12/SDF1alpha induced AKT activation. Accordingly, the migratory ability of SW480 cells towards CXCL12 is significantly reduced when NRP-2 function is blocked by siRNA or Semaphorin 3F. Indeed, in human tissue samples NRP-2 expression is significantly increased in lymph node metastases of colon cancer patients compared to the primary tumor.

In conclusion, NRP2 modulates the CXCR4 signaling axis in colorectal cancer cells. This axis might be one important player in the formation of regional lymph node metastases. Therefore, blocking NRP2 might to be a promising therapeutic avenue in treating colorectal carcinoma.

This project has been funded by a “Else-Kröner-Promotionsstipendium” to Hannah Schneider.

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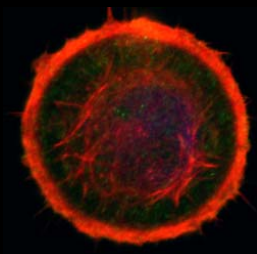
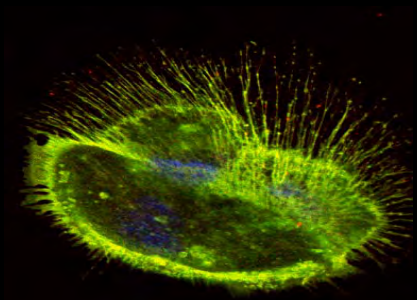


SESSION 6

(Thursday - 10/Oct/2013, 10:10 - 11:40 am)

Emerging Technologies



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Chairs:

Wolfgang Beisker, Helmholtz Zentrum München, Germany
Antje Dietrich, OncoRay, TU Dresden, Germany

Since many decades, progress in cytometry and cell-based analyses, respectively, has eminently profited from the fruitful interaction between academia and industry. This session shall provide an environment to present and discuss new methodological developments and emerging technologies which are likely to become important tools in ongoing and future-oriented cell and tissue research.

Fast Detection of Protein-Protein Interactions with an Automated FRET-based System on the Flow Cytometer

Kerstin von Kolontaj[1], Martin Büscher[1], Gabor Horvath[2]
[1]Miltenyi Biotec GmbH, Germany; [2]Institute of Innate Immunity, University Hospital, University of Bonn, Germany

As protein-protein interactions play a major role in almost all biological functions, much research is spend in this field. Conventional investigation methods, like the yeast two-hybrid system are highly time consuming. Foerster resonance energy transfer (FRET) is often used to identify protein-protein interactions by confocal microscopy. However, this requires expert knowledge and produces vast amounts of data.

In order to overcome those limitations, we developed a program, which automatically measures and calculates FRET efficiency on cell by cell basis on the MACSQuant flow cytometer. Relative signal changes of donor and acceptor fluorochromes are registered, therefore FRET efficiency can be determined very accurately. This allows the identification of protein-protein interactions on large cell numbers in a minimum of time, in high throughput screenings and is easy to use.

The program was built on the cell surface CD3-CD4 protein-protein interactions of T helper cells. We observed an increase in FRET efficiency after T cell activation due to the clustering of CD3 and CD4, followed by a decrease in FRET efficiency caused by CD3 and CD4 coreceptor internalization. For this test we used the VioBlue-FITC FRET pair.

To validate the program, we tested standard beads for GFP and mCherry FRET with defined linker length. FRET efficiency outputs of the program were compared to established manual FRET calculations. Due to the high correlation between those two methods (R2=0.9957) we could prove the functionality of this program.

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Scott Charles Cribbes
Brooks Life Science Systems, United Kingdom

The Celigo® Cell Cytometer from Brooks is a high throughput, multi application instrument offering unrivalled bright field and fluorescence imaging of a variety of microplates formats (1-well to 1536-well plates). The proprietary optics provide uniform bright field illumination throughout the entire well enabling the identification of cells anywhere in the well even right up to the edge. The Celigo is not restricted to cell based assays it can also be used for analysis of beads, large 3D objects like Embryoid Bodies and Tumour sphere as well as multi-cellular organism like C.elegans and zebra fish. Celigo's multi channel fluorescence in combination with flow cytometric like gating software provides rapid quantitative analysis of fluorescent cells with a variety of pre-packaged or user-defined assays. The Celigo provides image analysis usually only seen with pricey & complex high content imaging systems and with unrivaled simplicity & ease of use; it also delivers data acquisition at speeds usually only seen by HTS plate reading machines. All these attributes make the Celigo a flexible system that combines the successful attributes of flow cytometry and imaging in one easy to use, robust system.

Chromocyte: An Online Resource for the Flow Cytometry Community

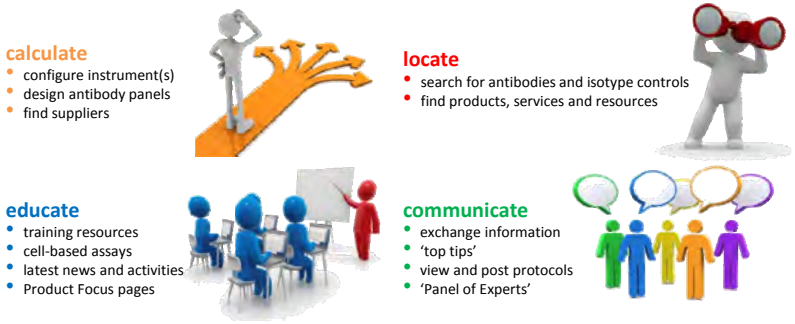
Alan Graham Pockley[1,2]
[1]Nottingham Trent University, United Kingdom; [2]Chromocyte Limited, Sheffield, United Kingdon

Associate Director, John van Geest Cancer Research Centre, Nottingham Trent University, Nottingham, UK and Founder and CEO, Chromocyte Limited, Electric Works, Sheffield, UK

The increasing complexity of flow cytometry instrumentation and the move towards customizable and 'non-standard' configurations have prompted the need for resources that allow flow cytometrists to search for antibodies that are suitable for their own particular instrument, identify the suppliers of these in a timely manner, and provide a comprehensive listing of flow cytometry-related material and resources. Chromocyte (www.chromocyte.com) has been developed as such a resource.

Chromocyte is a free online global resource which comprises four principal environments: CALCULATE, LOCATE, EDUCATE and COMMUNICATE.

Using a series of dropdown menus in CALCULATE, users can select and configure their instrument, and registered users can save these configurations. Instrument configurations that have been provided by Core Directors can be made available to other Users via Chromocyte's 'Facility Management' interface. Once configured, the specificity of antibodies required can be defined using a search tool which returns the maximum number of antibody-fluorochrome conjugates that are consistent with the configured instrument. Antibodies can be selected from this panel and the suppliers of these provided. Results can be downloaded as a spreadsheet which includes links to the



published literature which has used these products.

Chromocyte's guiding principle is to enhance the practice of flow cytometry and facilitate the adoption of this technique into laboratories that have no, or limited, prior experience of the technique. The resource is free to use due to the invaluable financial support of our Corporate Sponsors.

Kaluza for Gallios: A New Acquisition Software

Michael Braun
Beckman Coulter, Inc. Indianapolis, IN and Miami, FL

Kaluza for Gallios from Beckman Coulter employs cutting edge technology to bring flow cytometry within reach of more users than ever, from the least experienced to the most. Kaluza for Gallios is built on the strong, innovative foundation of Kaluza Analysis Software. The software excels with a simplified user interface, visual management and intuitive controls. It therefore is easy to learn, easy to use, and makes setting up and running complex multicolor flow experiments simpler than ever.

Kaluza for Gallios is currently in development.
For research use only. Not for use in diagnostic procedures.

UNCLE DOC - Automatic Analysis of Multi-Parameter Flow Cytometric Data Using an UNsupervised CLustering Engine

Martin Büscher
Miltenyi Biotec GmbH, Bergisch-Gladbach, Germany

In modern multiparameter flow cytometry, massive amounts of high dimensional data-sets are created. It turns out that the data-analysis step is a highly subjective and labor intensive time consuming process. In order to automate and standardize this process a clustering/cluster-analysis algorithm has been developed and tested on eight-paramter immunophenotyping data-sets. The results have been compared with manual analysis –carried out by experts- and the performance of the algorithm has been demonstrated.

In Vivo Multicolor Cellular Imaging for Translational Research

Hedi Gharbi
MAUNA KEA TECHNOLOGIES, Paris, France

Since its inception in the field of endomicroscopy, several years ago, fiber based, or probe based, confocal endomicroscopy (pCLE) has extensively proven the benefit of *in situ* & realtime examination of living tissues at the microscopic scale.

The constant efforts devoted by Mauna Kea Technologies at increasing image quality, reducing invasiveness or improving system's ergonomics have turned pCLE not only into an irreplaceable research and diagnosis instrument, but also an accurate decison making tool in the clinics, either in GI endoscopy or pulmonology.

A straightforward extension of pCLE approach was thus multicolour endomicroscopy, where simultaneous multi-laser excitation, again coupled with minimally invasive and microscopic resolution thin and flexible optics, not only brings complementary and valuable biological informations, but also paves the way to a combination of morphological and functional imaging.

We will present you a new system, Cellvizio Dual Band, capable of video rate (12 fps) *in vivo* & *in situ* fluorescence imaging with a microscopic resolution (1.4 µm). In its standard configuration, the system simultaneously operates at 488 and 660 nm, where it automatically performs the necessary spectral and photometric calibrations and where it provides unambiguously co-registered images, in realtime. The main hardware and software features (laser power control, sensitivity adjustment, image fusion, data exports) will be presented as well as a panorama of its current applications, illustrated with recent results, both in the field of preclinical and clinical imaging. A particular attention will also be paid to surgical applications, where fluorescence imaging at macroscopic and microscopic scale is now possible for intraoperatively targeting and characterizing suspicious areas.

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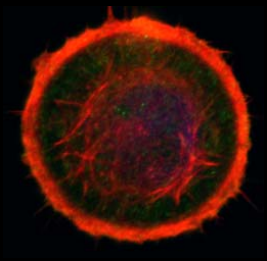
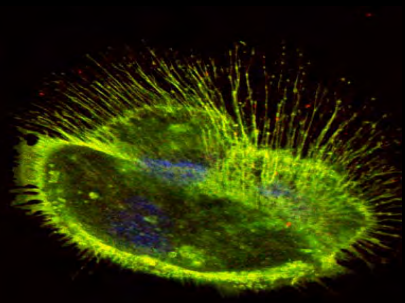


SESSION 7

(Thursday - 10/Oct/2013, 01:20 - 03:00 pm)

Monitoring (Malignant) Disease & Therapy Responses



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OncoRay National Center for Radiation Research in Oncology Dresden

Chairs:

Thomas Kroneis, Medical University of Graz, Austria
Gabriele Multhoff, TU Munich, Germany

Monitoring therapy is one of the key issues in clinical every-day life since ages. Due to emerging technologies and the advent of personalized medicine therapy monitoring is one of the current major fields in basic and translational cancer research. In this session we will be given an update on circulating tumor cell analysis as one example of disease monitoring that already proved to work in particular settings. On the other hand flow cytometry reaches out to disease monitoring as well. Hence, the session will also cover current activities in monitoring minimal residual disease by flow cytometry with respect to hematologic diseases. Furthermore, the session will provide room for additional contributions in the field of single and rare cell analysis and therapy monitoring, respectively.

Circulating Tumor Cells: Current State and Future Perspectives

Sabine Riethdorf, Klaus Pantel
Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Microscopic spread of cancer cells is usually undetected by current imaging technologies. Therefore, a variety of sensitive methods have been developed to detect circulating tumor cells (CTC) in the peripheral blood. The capture of CTC that are surrounded by millions of blood cells is based on the physical and/or biological properties of cancer cells. Thus far, only the automated CellSearch™ system has been cleared by the FDA. However, many assays including the CellSearch™ system rely on EpCAM for CTC capture and cytokeratins for CTC detection but these epithelial marker proteins can be downregulated during epithelial-mesenchymal transition. Besides assessing the clinical utility of CTC for assessment of prognosis, monitoring of CTC during and after systemic therapy can provide unique information for the clinical management of individual cancer patients. In particular, the molecular analysis of CTC as “liquid biopsy” will give new insights into therapeutic targets and the selection of tumor cells under specific therapies. In addition, molecular and functional characterization of CTC opens a new avenue for understanding metastatic spread of tumor cells with important implications for future therapies. Implementation of CTC analyses in clinical trials testing new anti-cancer agents as companion diagnostics will speed up the cumbersome and expensive drug validation process in oncology.

Diagnostic Profiling of Single Circulating Tumor Cells

Bernhard Polzer,
Personalized Tumor Therapy, Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM-R), Regensburg, Germany

Worldwide more than 40,000 patients are included into clinical trials that explore the implementation of circulating tumour cell (CTC) analysis into therapy decisions. However, workflows are lacking that enable standardized routine application. Particularly, molecular single cell analyses form a sine qua non for many targeted therapies. We combined CTC enrichment and detection by the widely available CellSearch® method, pure single cell isolation by the DEPArrayTM system and a non-random whole genome amplification method (WGA; *Ampli1* TM) for single cells and applied it to 525 CTC and 189 leukocytes of 69 breast cancer patients. We defined morphological and molecular criteria for intact CTC calling and determined quality-control criteria for single CTC DNA to assess point mutations, gene amplifications and genome wide copy-number changes. Genome integrity after fixation, storage and processing is significantly lower in CTC than in identically treated white blood cells (WBC) and freshly isolated and unfixed WBC (37% vs. 66% vs. 94%, respectively; $p < 0.0001$). We could detect high abundancy of PIK3CA mutations in CTC of breast cancer patients (35.4%), and frequent ERBB2 DNA amplification in CTC of patients initially classified as HER2-negative by analysis of the primary tumor. Additionally, we carefully assessed cell-to-cell heterogeneity in selected patients by aCGH. In conclusion, we hypothesize that to elevate CTC analysis to the state of a diagnostic liquid biopsy, CTC enrichment and detection assays should be complemented by careful selection of cells with high genomic integrity for molecular analysis.

Dissecting Cell Cycle Control and Proliferation on the Cellular Level – Lessons from the Survivin-RNAi-Phenotype

Hanns Achim Temme, Ralf Wiedemuth, Gabriele Schackert
Department of Neurosurgery, Medical Faculty Carl Gustav Carus, TU Dresden, Dresden, Germany

A likely candidate for an RNAi therapy of cancer is the inhibitor of apoptosis protein (IAP) Survivin. Yet, it is now unanimously recognized that the another molecular function of Survivin is linked to the control of the spindle assembly checkpoint (SAC) and cytokinesis In addition, several lines of evidence point to a role of Survivin in connecting mitosis with control of cell cycle arrest. However, the mechanism how Survivin controls the cell cycle has remained elusive. In this study, we assessed the effects of Survivin-RNAi on the cell cycle in HCT116, MCF7 and U87-MG and their isogenic p53-deficient cell lines. In all cell lines tested stable knock down of Survivin did not cause instant apoptosis but mitotic defects characterized by multipolar spindles and polyploidy. Further Western Blot analysis showed a time-dependent increase of markers of G1 cell cycle arrest (i.e. p21waf/cip, CyclinD1) in p53-positive cells after knock down of Survivin which was accompanied by the development of cells bearing genomic DNA contents in the range of 4N to 32N. The question why p53-positive cells with knock down of Survivin and displaying molecular signs of a G1 cell cycle arrest were still capable to replicate their genomes was unraveled using flow cytometry. Our results evidently show that a transient activation of p53 and increase in p21waf/cip after loss of Survivin function is connected to its role in controlling proper mitosis.

Monitoring of Minimal Residual Disease (MRD) in Malignant Hematologic Diseases by Flow Cytometry

Uta Oelschlägel
Medical Clinic I, Medical Faculty Carl Gustav Carus, TU Dresden, Germany

Flow cytometry is reported to be one possibility for monitoring of MRD during disease course in hematologic malignancies. Therefore, the detection of a characteristic disease related antigen pattern at diagnosis is prerequisite.

Thus, depending on the subtype of disease, different abnormalities could be detected: in acute leukemias: aberrant expression of lymphocytic (e.g. CD5 in AML) or myeloid antigens (e.g. CD13 in ALL), asynchronous antigen expression (e.g. $CD11b^{+}/CD34^{+}$); MDS: a combination of abnormal differentiation patterns of granulo- and monocytopoiesis and aberrant expression of lymphocytic antigens (e.g. in MDS with deletion 5q); B-cell-lymphoma: specific antigen patterns, e.g. $CD19^{+}/CD5^{+}/CD79b^{-}/CD20^{dim}$ in B-CLL or $CD38^{++}/CD138^{+}/CD56^{+}/CD19^{-}/CD45^{-}$ in multiple myeloma (MM) or detection of aneuploidy in simultaneously immunophenotyped cells; PNH: detection of cells with deficiency of GPI-anchored antigens on different cell lineages (granulo-, mono- and erythropoiesis).

Problems caused by a possible „antigen shift“ during therapy could be minimized by monitoring not only one single antigen abnormality. Thus, multi-parameter analyses measuring large amounts of cells (at least 100,000 cells for a sensitivity of 0.1%) should be performed. Furthermore, reproducible instrument setting over longer time periods are obligatory, because of the evaluation of abnormal fluorescence intensity of malignant vs. normal cells. Different cut-off values should be applied at different time points of therapy, especially in acute leukemias.

Besides high sensitive PCS analyses, flow cytometry could be a useful tool in disease monitoring of malignant hematologic diseases, taking into account all the above mentioned prerequisites.

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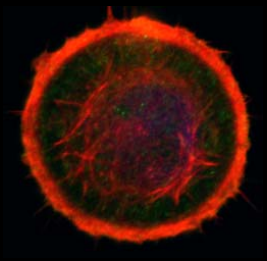
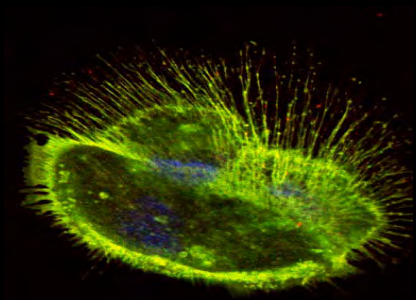


SESSION 8

(Thursday - 10/Oct/2013, 03:30 - 04:30 pm)

Cutting-Edge Animal Models of Disease



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OncoRay National Center for Radiation Research in Oncology Dresden

Chairs:

Anja Wege, University of Regensburg, Germany
Georg Breier, TU Dresden, Germany

Imaging and cytometric analyses are essential tools in mechanistic and treatment-related *in vivo* studies. Small animal models are integral to many translational research activities in regenerative medicine, oncology, and numerous other disease-related studies. However, one has to be aware of the challenges and limitations of the model chosen for approval of a specific biomedical hypothesis. Despite using knock-out and knock-in animal models to study the impact of specific genes and gene products in developmental processes and disease, immune-deficient models are frequently applied to allow the implementation of human cells and tissues in an *in vivo* environment. The session will highlight some relevant cutting-edge animal models for human cell monitoring which are expected to be of great interest when combined with advanced *in vivo* imaging and cytometric methodologies. In particular, we will discuss the usefulness of humanized mice models and human cancer xenograft models in zebrafish.

Zebrafish Xenografts as a Tool for *in vivo* Studies on Human Cancer

Martina Konantz, Claudia Lengerke
Department for Hematology and Oncology, University of Tuebingen Medical Center, Tuebingen, Germany

The zebrafish has become a powerful vertebrate model for genetic studies of embryonic development and organogenesis. Zebrafish facilitate the performance of reverse and forward genetic approaches, including mutagenesis and small molecule screens. More recently, zebrafish have been used for studies on human cancer. Several publications report the feasibility of xenotransplanting human cancer cells into zebrafish embryos and adult fish. Main advantages of the model are the short incubation times, the possibility to monitor tumor-induced angiogenesis, invasiveness, and response to a range of treatments *in vivo* and in real time as well as to easily perform limiting dilution assays to detect frequencies of cancer stem cell populations displaying tumor-initiating properties. Major drawbacks are the more profound differences to human niches and growth factor environments in zebrafish compared to the classical xenotansplant assays performed in mice which may limit validity and translational utility of the zebrafish model.

In summary, xenotransplantation into zebrafish provides an exciting model for the *in vivo* exploration of specific aspects of human cancer cell biology complementing classical studies in mice. Further side-by-side comparative studies are needed to explore the validity of results obtained in zebrafish versus murine xenotransplant studies.

Humanized Tumor Mice - New Concepts for Translational Research

Anja Wege[1], Katharina Schardt[2], Olaf Ortmann[1], Ernst Michael Jung[3], Alexander Krömer[4], Gero Brockhoff[1]

[1]Clinic of Gynecology and Obstetrics, Caritas Hospital St. Josef, University of Regensburg; [2]Institute of Pathology, University of Regensburg; [3]Institute of Radiology, University of Regensburg; [4]Department of Surgery, University of Regensburg, Regensburg, Germany

The immunological impact on antibody based anti-cancer therapies remains incompletely understood due to the lack of appropriate animal models for in-vivo analysis. To overcome this limitation, we generated a novel humanized tumor mouse (HTM) model by concurrent transplantation of human hematopoietic stem cells and human breast cancer cells in neonatal NOD-scid IL2 receptor null mice. Five weeks after intrahepatic transplantation a functional human immune system developed in all organs and in addition tumor cells were detectable in lung and bone marrow (early dissemination). In the age of three months, solid tumors associated to liver (and spleen) or tumor cell effusions were detectable. Interestingly, BT474 cells (originally isolated from a solid tumor) resulted in solid tumor formation and weak dissemination whereas SK-BR-3 originally isolated from pleural tumor effusion reappeared in tumor ascitis accompanied by metastases in these mice. High resolution ultrasound (HRU) in combination with contrast enhanced ultrasound (CEUS) and color-coded elastography enabled the early detection and characterization of small liver lesions in HTM *in vivo*. Tumor growth was accompanied by specific T cell maturation and tumor cell specific T-cell proliferation. In addition, Natural Killer cell accumulation and activation was observed in HTM which was further enhanced upon IL-15 treatment facilitating the possibility of immune cell modulation in, e.g. antibody-dependent cell-mediated cytotoxicity (ADCC) based immunotherapeutic approaches in HTMs. Furthermore, T cell receptor (TCR) analyses indicated a nearly full human TCR repertoire in spleen, liver and lymph nodes including some dominantly expressed TCR chains.

The neonatal transplantation enabled the combined transfer of major histocompatibility complex (MHC) mismatched tumor cells together with human hematopoietic stem cells resulting in a solid coexistence and interaction without evidence for rejection. Overall, humanized tumor mice represent a powerful *in-vivo* model that permits the investigation of the human immune defence against cancer, in particular with respect to antibody based targeted therapies and might reveal new strategies to overcome therapy failure in non responder patients.

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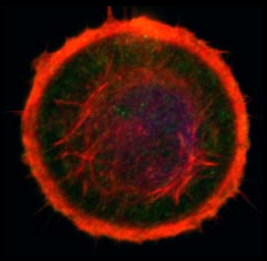
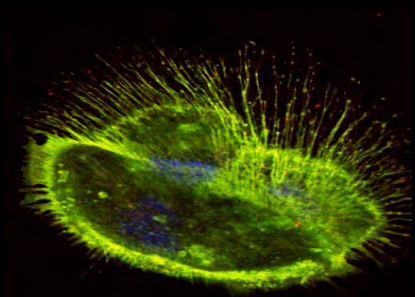


SESSION 9

(Thursday - 10/Oct/2013, 04:30 - 05:30 pm)

Klaus-Goerttler-Session
Microbiology beyond Biomedicine



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OncoRay National Center for Radiation Research in Oncology Dresden

Chairs:

Susanne Günther, Helmholtz Centre for Environmental Research - UFZ Leipzig, Germany
Lars M. Blank, RWTH Aachen, Germany

The first part of this session will focus on the applications of flow cytometry in microbiology. Microorganisms are widely used for the production of valuable products ranging from energy (hydrogen/methane) to various chemicals. Where in pure cultures the main problem for utilization of microbial activity is population heterogeneity, in mixed cultures it is the limited knowledge about individual microorganisms and their interaction within the ecosystem. Flow cytometry in combination with omics techniques has been successfully applied for the investigation of population heterogeneity and the investigation of natural communities without cultivation.
The second part will be the presentation of this year's Klaus Goerttler award to a younger scientist with an excellent scientific work related to the wide field of cytometry.

Industrial Biotechnology - Can Cytometry Help to Meet the Challenges?

Thomas Bley
Institute of Food Technology and Bioprocess Engineering,
TU Dresden, Dresden, Germany

The increasing demand for a sustainable supply of food, raw materials and fuels, together with recent scientific progress, is the major economic driving force behind growth of the Knowledge Based Bio-Economy (KBBE). The bioeconomy – the sustainable production and conversion of renewable biomass for a range of food, health, fibre and industrial products and energy – can play an important role in both creating economic growth, and formulating effective responses to pressing global challenges. In this way it contributes to a smarter, more sustainable and inclusive economy.
Industrial biotechnology ("White biotechnology") realized typically in biorefineries is one of the crucial keys for realizing the change from an economy based on fossil raw materials to a sustainable bioeconomy.
Constructing and controlling bioprocesses in industrial plants needs information about the biocatalytic processes in the reaction system. Traditionally the so called indirect measurement concept is applied. That means that the results of microbial life like oxygen consumption or carbon dioxide production are used for parametrizing bioprocess models.
Cytometry now opens the door for looking into the cell population, for a direct observing of the behaviour of the microbial populations. This information out of "glassy" cells is the basis for increasing the economic efficiency of most bioprocesses.
This is demonstrated by some examples:
Control of feeding strategy for producing hydrophobins by recombinant yeasts
Determining the biomass concentration in solid state fermentations with fungi
Optimizing the production of secondary metabolites by plant cell *in vitro* cultures
Optimizing the production of phycobiliproteins by cyanobacteria
The development of the technique of flow cytometry has now achieved a status, where the devices are robust, easy to use, and reasonably priced. This is the starting point for a broad application in industrial biotechnology - to gain knowledge about cells in bioprocesses for fulfilling a Knowledge Based Bio-economy.

References

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- Müller, S., Harms, H., Bley, Th., Origin and analysis of microbial population heterogeneity in bioprocesses, Current Opinion in Biotechnology, 21 (2010) 100-113
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Flow Cytometry of Archaea

Rolf Bernander
Department of Molecular Biosciences, Stockholm University, Sweden

Our work is focused on the cell cycle of organisms from the third domain of life, Archaea. We use flow cytometry to characterize the general organization of the cell cycle in a wide range of species, including hyperthermophiles, methanogens and low-temperature isolates. We also use the technique to investigate effects of drug treatments and mutations, to compare DNA content and cell mass in exponential and stationary phase cultures, to monitor cell cycle progression in synchronized populations, and to confirm that cells reside in S phase during replication origin mappings. An overview of our flow cytometry-based applications will be provided.

Congradulations to our 2013 Klaus-Goerttler Awardee

Petra Bacher[1],
[1]Department of Cellular Immunology, Clinic for Rheumatology and Clinical Immunology, Charité - University Medicine Berlin

Mrs. Bacher receives the award for excellent scientific work during her PhD study, in particular the approaches leading to the development of a flow-cytometric assay for high sensitivity detection of rare antigen-specific $CD4^+$ T cells.

Two selected publications related to this work are:

Bacher P, Schink C, Teutschbein J, Kniemeyer O, Assenmacher M, Brakhage AA, Scheffold A. Antigen-reactive T cell enrichment for direct, high-resolution analysis of the human naïve and memory Th cell repertoire. J Immunol. 2013 Apr 15;190(8):3967-76.

Bacher P & Scheffold A. Flow-cytometric analysis of rare antigen-specific T cells. Cytometry A. 2013 Aug;83(8):692-701.

The awardee will present her work in a short talk and on a poster (see Abstract P11) entitled

Antigen-Reactive T Cell Enrichment for Direct, High Resolution Analysis of the Human Naïve, Memory and Regulatory $CD4^+$ T Cell Repertoire

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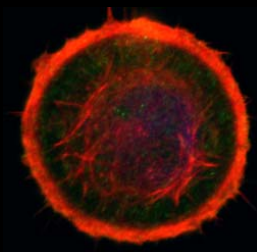
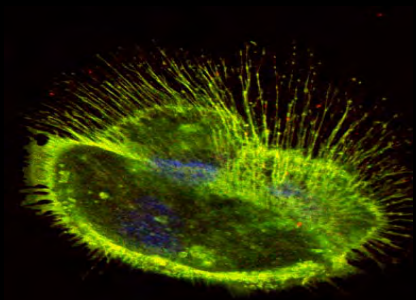


SESSION 10

(Friday - 11/Oct/2013, 09:00 - 10:30 am)

Nanobiotechnology/ Nanobiomedicine



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Chairs:

Wolfgang Fritzsche, IPHT Jena, Germany
Ulrike Taylor, FLI Mariensee, Neustadt, 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.

Conjugated Gold Nanoparticles as Selective Imaging Tools in Cell Biology

Stephan Barcikowski
Technical Chemistry I and Center for Nanointegration Duisburg-Essen (CENIDE), University of Duisburg-Essen, Germany

Gold nanoparticles (AuNPs) are suitable tools for bioimaging as they show a good biocompatibility and in contrast to organic dyes they do not suffer from photobleaching. However, in order to selectively bind to target sites and, for intracellular targeting, to penetrate cell membranes they need to be functionalized with biomolecules. In some cells peneration is favored by small particle sizes (5 nm). Since optical imaging with confocal microscopy requires larger particles > 60 nm, imaging strategies may include NP aggregation, which results in an optically detectable plasmon shift. In this presentation we describe the development of bioconjugated AuNPs as bioimaging tools for sperm sexing experiments including cell penetration and DNA hybridisation of the nano probe as well as toxicity aspects.

Toxic Effects of Silver Nanoparticles on Neural Cells: Uptake, Oxidative Stress and Acute Calcium Responses in Primary Mixed Neural Cell Cultures

Georg Reiser
Institute for Neurobiochemistry, Medical Faculty,
Otto-von-Guericke-University Magdeburg, Germany

Because of their antimicrobial, optical, and catalytic properties silver nanoparticles (SNP) have gained particular interest for many commercial applications. According to the Woodrow Wilson inventory approximately 30% of all nanoparticle enabled products contain nanosilver. Thus SNP are highly commercialized and are now being used in many daily-life products. In the body, nanoparticles can be systemically distributed and then may affect secondary target organs such as the central nervous system (CNS). Nanoparticles can reach the CNS via different routes. Putative adverse effects on the CNS are rarely investigated to date. Here we studied a mixed primary cell model consisting mainly of neurons and astrocytes and a minor proportion of oligodendrocytes to analyze the effects of well-characterized 20 nm and 40 nm silver nanoparticles (SNP). Similar gold nanoparticles served as control and proved inert for all endpoints tested. SNP induced a strong size-dependent cytotoxicity. Additionally in the low concentration range (up to 10 µg/ml of SNP) the further differentiated cultures were more sensitive to SNP treatment. For detailed studies, we used low/medium dose concentrations (up to 20 µg/ml) and found strong oxidative stress responses. Reactive oxygen species (ROS) were detected along with formation of protein carbonyls and the induction of heme oxygenase-1. We observed an acute calcium response, which clearly preceded oxidative stress responses. ROS formation was reduced by antioxidants, whereas the calcium response could not be alleviated by antioxidants. Finally, we looked into the responses of neurons and astrocytes separately. Astrocytes were much more vulnerable to SNP treatment compared to neurons. Consistently, SNP were mainly taken up by astrocytes and not by neurons. Immunofluorescence studies of mixed cell cultures indicated stronger effects on astrocyte morphology. Altogether, we can demonstrate strong effects of SNP associated with calcium dysregulation and ROS formation in primary neural cells, which were detectable already at moderate dosages. The high vulnerability of astrocytes towards nanoparticle exposure, as demonstrated here, might thus have fundamental consequences on the proper function of neural networks.

Nanoplasmonics in Biomedicine: From Thermodynamics at the Nanoscale to Remote Release from Polymeric Capsules, Lipid Systems, Red Blood Cells

Andre Skirtach
Department of Interfaces, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Applications of nanotechnology in biomedicine and biotechnology is promising to both explore matter at the nanoscale and generate advances in these fields. Nanoplasmonics encompasses laser interaction with noble metal nanoparticles and exploits phenomena associated with local temperature rise around nanoparticles. We look into local heat generation and thermodynamics at the nanoscale, phase transition of polymer and lipid systems in the quest to understand the basics of these interactions. Subsequently, this understanding is essential to apply these phenomena in biomedical and biotechnology: remote release from a variety of carriers ranging from polymeric to lipid systems and red blood cells will be discussed. We also look into applying this system to shine light on fundamental processes in immunology.

Labeling of *Staphylococcus aureus* with Fluorescent or Para-magnetic Nano-particles Highlights new Capabilities for Following Host-Pathogen Interactions

Kristin Surmann[2], Maren Depke[1], Petra Hildebrandt[1], Nico Jehmlich[2], Stephan Michalik[1], Sarmiza E. Stanca[3], Wolfgang Fritzsche[3], Uwe Völker[2], Frank Schmidt[1]

[1]ZIK FunGene Applied Poteomics, Universitätsmedizin Greifswald; [2]Functional Genomics, Universitätsmedizin, Greifswald; [3]Institute for Photonic Technologies (IPHT), Jena, Germany

Severe infections caused by pathogens such as *Staphylococcus aureus* are still a major issue. The rapid development of resistance to antibiotics increases the need to find successful strategies for prevention and therapy. Therefore, a comprehensive understanding of processes during interaction of host and pathogen is crucial.

Modern microscopy and OMIC's techniques enable tracing of trafficking during internalization and molecular characterization of infection processes. However, proteomic analysis of the adaptation of a pathogen to the host environment is often limited by the low number of bacteria available from infected host cells. Therefore, several techniques were established to enrich bacteria such as cell sorting using fluorescent proteins like the green fluorescent protein or immuno-magnetic approaches.

However, when investigating e.g. clinical isolates suitable antibodies are not always available and labeling with fluorescent proteins is not always possible and can lead to artificial physiological changes in the original isolate.

Here, we introduce a technique which can be generally applied for studies of infection models using gold- (Au) or ferric oxide-core (FeOx), poly(vinyl alcohol) coated, fluorescence-labeled nano-particles (NP). They can be used to investigate replication and localization by fluorescence microscopy, or for isolation of the fluorescence pre-labeled pathogen from the host using cell sorting. Due to its para-magnetic properties, the FeOx-NP can further be used to separate labeled pathogens with the help of a strong magnetic field.

To test the applicability of this approach *S. aureus* HG001 cells were labeled with Au-NP or FeOX-NP. More than 80% of the bacteria were labeled already after 15 min. In a stable isotope labeling of amino acids in cell culture (SILAC) based mass spectrometry analysis, specific patterns of proteomic changes caused by labeling were not detected after 24 h of incubation. Further, we infected the human bronchial epithelial S9 cell line with pre-labeled S. aureus to investigate early adaptation of the bacteria after 2.5 h and 4.5 h post-infection.

Therefore, the Au-core NP were used to sort bacteria from host cell debris and as a result about 300 proteins were identified from less than $5*10^5$ bacteria. After magnetic enrichment using the FeOx-NP we could identify about 400 proteins of S. aureus in the same infection model settings.

With our approach we provide an easy, fast, and cost-effective method which allows investigation of host-pathogen interactions by fluorescence microscopy and proteomics by enrichment of NP-labeled bacteria which could be easily applied for work with other pathogens and host models.

23rd Annual Conference of the German Society for Cytometry

October 9th-11th 2013
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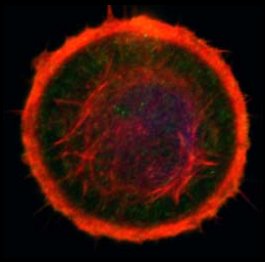
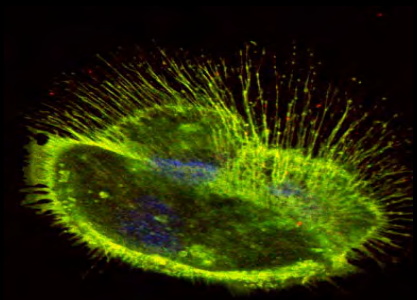


SESSION 11

(Friday - 11/Oct/2013, 11:00 - 12:30 am)

Cytometry meets Bioinformatics



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Susann Müller, Helmholtz Centre for Environmental Research - UFZ Leipzig, Germany
Frank Schmidt, University of Greifswald, Germany

Using flow cytometry to measure, evaluate, and interpret the dynamics of various cell types and their function in highly diverse and complex cell systems is challenging. One drawback is the limited accession to reliable cytometric pattern analysis. E.g., until now pattern analysis most often requires individual and manual gating decisions which is time consuming and results in person dependent data evaluation. Expertise is needed to gate sub-sets of cells regarding specific cell characteristics. However, also gating independent strategies are already on the market like the FlowFP package that uses a kind of probability binning. In addition, flow cytometry is often combined with other omics techniques which further increase the information that can be obtained from subsets of cells in a cell system.
In this session we aim to present and discuss bioinformatic tools or ideas that may be able to handle huge cellular data sets and that may lead to an integrated systems analysis.

Image-Based Quantification of Cellular Dynamics

Ingo Roeder
Institute for Medical Informatics and Biometry (IMB),
Medical Faculty Carl Gustav Carus, TU Dresden, Germany

The continuous analysis of individual cell fates within a population of cells is still a major experimental challenge. However, recent developments in high-resolution time-lapse video microscopy now facilitate the tracking and the quantification of cellular dynamics on the single cell level. The obtained information on cellular motion, spatial organisation, divisional history, or differentiation of cells and cell ensembles, can be analysed using different representations, such as cellular flows or genealogies. To go beyond a pure descriptive analysis of this type of data and to statistically extract reliable information about effecting variables and control mechanisms, which underlie the patterning and the fate decisions of cells, it is necessary to analyse large numbers of cells. This, in turn, requires the development and application of automatic cell tracking algorithms.

In the talk I will present computational methods that allow for the automatic segmentation (recognition) and tracking of cell ensembles and individual cells and, based hereon, the reconstruction and analysis of behavioural characteristics and cellular genealogies from time-lapse video data. These methods will be discussed with respect to their application in two biological systems, namely the haematopoietic stem cell niche and the developing zebrafish embryo.

OmicsData and Visualization – What's in the haystack?

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

From raw data to gene or protein expression profiles, from cell populations to complex cultures, currently gene or protein expression analysis works with a variety of differently structured data. Although data visualization is closely connected with data analysis approaches; this talk will focus solely on integrated data visualization. By complementing the traditional tools such as bar charts or line graphs a tool kit of new sophisticated visualization techniques became available during the last decade. Many concerns regarded to the display of single but also complex data will be explained and discussed. New visual approaches and applications will be introduced.

Revealing Regulatory Principles of Functional Heterogeneity in Haematopoietic Progenitor Cells: a Combined Experimental and Modelling Approach

Enrica Bach[1], Thomas Zerjatke[2], Manuela Herklotz[3], Nico Scherf[2], DietgerNiederwieser[1], Ingo Roeder[2], Tilo Pompe[3,4], Michael Cross[1], Ingmar Glauche[2]

[1]Universität Leipzig, Department of Hematology, Oncology and Hemostasiology; [2]Technische Universität Dresden, Institute for Medical Informatics and Biometry; [3]Leibniz Institute of Polymer Research Dresden, Max Bergmann Center of Biomaterials; [4]Universität Leipzig, Institute of Biochemistry, Germany

Cultures of haematopoietic stem and progenitor cells are often characterised by heterogeneous appearance, proliferation, background differentiation and cell death, and the maintenance of self-renewal ability within a small subpopulation of cells only. The mechanisms that maintain the fraction of self-renewing cells in a continuously expanding, intrinsically heterogeneous population are unknown, but are likely relevant to similar homeostatic processes *in vivo*.

Using the well-established, multipotent murine progenitor cell line FDCP-Mix we present a combined experimental and mathematical modelling approach to investigate population-intrinsic heterogeneity. We measured turnover, differentiation, and self-renewal of the cells at both the population and single cell levels and adapted an established single-cell based model of HSC organisation to interpret the experimental results. Constriction of the wide variety of model scenarios to a single model refinement was only achievable by the successful application of long-term single-cell tracking and the statistical assessment of correlation structures within the resulting cellular genealogies.

The observed heterogeneity is best explained by a combination of differential regulation of cellular turnover and apoptosis during background differentiation along different lineages. Our results demonstrate the power of a combined experimental/modelling approach in which single cell fate analysis is the key to revealing regulatory principles at the cellular level.

A new Approach for Semiautomated Analysis of Multispectral Flow Cytometric Data

Kristen Feher, Jenny Kirsch, Andreas Radbruch, Hyun-Dong Chang, Toralf Kaiser
German Rheumatism Research Centre (DRFZ) Berlin, Germany

We demonstrate how a full staining can be combined with its respective Fluorescence-Minus-One (FMO) controls using multivariate projective methods. This results in the semi-automated identification of cell populations within that sample, without the need for manual gating or unsupervised clustering methods. Given that spectral overlap ('spillover') between fluorochromes is automatically accounted for in our method, staining panels no longer need to be so stringently optimized with respect to spillover, giving the experimenter a higher degree of flexibility in experimental design. As a consequence, fixed equally-spaced bandpasses can be used for all experiments regardless of staining panel choice, which greatly increases user-friendliness, as well as reproducibility between experiments by greatly simplifying calibration. As an outlook, we discuss how our method should facilitate the routine and cheap use of a high number of fluorochromes in the future.

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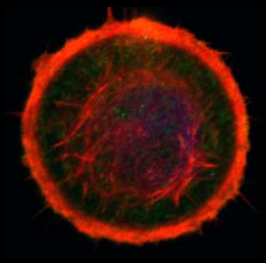
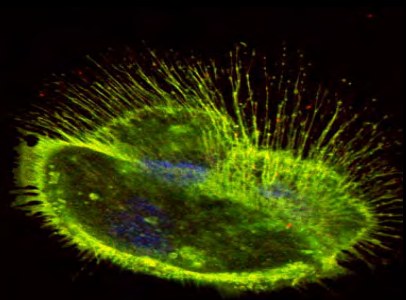


SESSION 12

(Friday - 11/Oct/2013, 01:30 - 02:30 pm)

Meet the Expert Lecture



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Leoni A. Kunz-Schughart, OncoRay, TU Dresden, Germany

Dr. Harris established the first cord blood bank in the USA in 1992. He currently serves as Director of the Cord Blood Stem Cell Bank, is member of the Arizona Cancer Center, the Children's Research Center, and the Arizona Arthritis Center. His research interests include stem cells and regenerative medicine, cancer research/stem cell transplantation and gene therapy. He is founder/co-founder of four companies.

The Future of Cord Blood Stem Cell Banking: Regenerative Medicine & Cellular Therapies

David Harris
College of Medicine, University of Arizona, Tucson, AZ, United States of America

The idea of stem cells as medical therapy has become almost commonplace. Millions of individuals have stored stem cells in biobanks for future use, and tens of thousands of patients have received stem cell transplants or infusions for regenerative medicine therapies. Stem cells have the capacity to generate new cells to replace those lost through injury, and have shown promise in animal models of neurological injury. This presentation will summarize the pre-clinical data for use of cord blood (CB) stem cells for the treatment of these conditions. In addition, case studies will be presented of multiple pediatric patients that have undergone autologous CB stem cell infusions to treat these conditions. Conditions affecting beneficial outcome will be discussed as well as uses of CB stem cells for other regenerative medicine applications. In addition, work will be presented on the creation of humanized mice using cord blood stem cells in order to study the mechanisms involved in these beneficial therapies.

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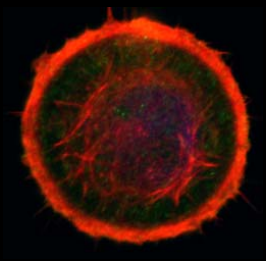
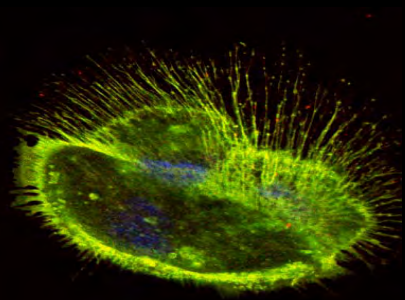


SESSION 13

(Friday - 11/Oct/2013, 02:30 - 04:00 pm)

Monitoring Immune Responses



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Chairs:

Hyun-Dong Chang, DRFZ, Berlin Germany
Karsten Kretschmer, CRTD, Dresden, Germany

Immune responses are a complex spatial and temporal interplay on many different cell types leading in the end to protective immunity, immunopathology, or chronic inflammation. Technological advances in cytometry allow us to monitor such immune responses or defects thereof through dynamic measurement of the cellular composition and function in blood but also in different organs. This enables us to gain a better understanding of the course of an immune response and the cellular and molecular correlates of immunity in health and disease. In this session we will give insights into novel technologies and recent results in immune monitoring with possible impact on diagnostics of disease, development of improved vaccination strategies, and immunomodulatory therapies.

Single Cell Raman Micro-Spectroscopy – a Powerful Tool in Clinical Diagnosis

Jürgen Popp
Institute of Physical Chemistry, Jena University, Germany

Within the last years a rapid increase of applications of Raman spectroscopy in particular to address biomedical questions has been observed. New concepts of cancer diagnostics towards a rapid identification of sepsis pathogens were among the most important questions, which were answered by innovative Raman approaches. Raman based methods are ideal tools for medical and life science research: Raman measures without contact, providing information on processes within living cells without disturbing them. Here we describe briefly some of our latest results concerning the application of Raman microspectroscopy to characterize prokaryotic and eukaryotic cells for medical diagnosis. The first part reports about an online / on-site identification of microorganisms based on their characteristic Raman fingerprint. We will show that such a rapid microbial analysis i.e. the identification of single bacterial cells without the need of pure cultures or any cultivation step is of great relevance for an efficient medical diagnosis (e.g. rapid identification of pathogens in urine samples), air- and soil monitoring (e.g. detection and identification of anthrax endospores embedded in complex matrices) or food analysis. Furthermore, we will report about the great potential of Raman spectroscopy for a label-free discrimination between normal and tumor cells based on their biochemical composition or towards establishing a Raman spectroscopic hemogram i.e. characterizing leukozytes. Thereby cellular Raman spectra were recorded after drying, in laser tweezers or trapped in a microfluidic environment (= biochip Raman cell sorting). In particular the implementation of Raman spectroscopy and optical traps in a microfluidic chip allows for Raman activated cell sorting which offers large potential for an automated classification of cells like e.g. circulating tumor cells.

Acknowledgements
Financial support of the EU, the ”Thüringer Kultusministerium”, the ”Thüringer Aufbaubank”, the Federal Ministry of Education and Research, Germany (BMBF), the German Science Foundation, the Fonds der Chemischen Industrie and the Carl-Zeiss Foundation are greatly acknowledged.

Reference Values of Leukocyte Subpopulation Counts of Men and Women Aged 18-87 Years. First Results from the Analysis of Healthy Adults in the Leipzig LIFE study.

Susanne Melzer[1,2], Jozsef Bocsi[1,2], Silke Zachariae[1,3], Christoph Engel[1,3], Markus Löffler[1,3], Attila Tárnok[1,2]

[1]Universität Leipzig: LIFE – Leipzig Research Center for Civilization Diseases; [2]Universität Leipzig: Heart Center Leipzig GmbH, Department of Pediatric Cardiology; [3]Universität Leipzig: Institute for Medical Informatics, Statistics and Epidemiology, Germany

Aim: The aim of this study was to present reference values for men and women between 17 and 87 years for frequent and rare leukocyte subpopulations.

Background: Deviations of lymphocyte subset counts from normal are commonly used for diagnosis of leukemia, HIV infection, or immune defects. In diverse studies, reference values are predominantly presented for children from birth to puberty or for adults that are older than 60 years. However, cell count values dependent on gender, age, ethnicity and life-style. Therefore, we want to present reference values of lymphocyte subpopulations for adults of a broad age range from 18-87 years.

Methods: 647 EDTA blood samples were collected from volunteers of the A1 LIFE study (m: 256, w: 391; age range: 18-87 years). All participants went through a detailed standardized medical examination program and questionnaires. Blood samples were lysed to exclude erythrocytes, stained with 13 antibodies against cell surface markers (CD45, CD3, CD4, CD8, CD14, CD19, CD25, CD127, CD38, CD69, CD16, CD56, HLA DR) dyed with 10 different fluorochromes and measured on a 10 color Navios flow cytometer (Beckman Coulter). Laser alignment was daily checked with Flow Check Pro and instrument sensitivity by Rainbow beads. Titration experiments, stability-tests of the utilized premixed antibody cocktail, and analysis of the intra-assay variance in repeated preparations of 11 aliquots were done. Data were analysed with Flowjo 7.6.4 using a manual, sequential gating strategy with a pre-defined analysis protocols. Subpopulations were manually gated and the reliability of data analysis was found to have a low bias and high accuracy by analyzing blinded samples by three trained cytometrists. Statistical analysis were performed by Student´s t-test, comparing males vs. females and young (30-45 years, n=105) vs. old (65-80 years, n=246) with statistically significant differences $p \leq 0.05$.

Results: In agreement with other studies total leukocyte count for all individuals was 5.75±1.41x109 cells/l with neutrophils: 3.64±1.25x109 cells/l; monocytes: 0.27±0.11x109 cells/l; lymphocytes: 1.70±0.61x109 cells/l; T-cells: 1.17±0.48x109 cells/l and B-cells: 0.23±0.12x109 cells/l.

Further subpopulations were distinguished as cytotoxic T cells: 0.20±0.13x109 cells/l ; T-helper-cells: 0.87±0.37x109 cells/l; Tregs $CD4^+CD127^{lo}CD25^+$: 69.19±41.34x106 cells/l; $CD4^+CD8^+$ T-cells: 8.34±6.43x106 cells/l; NKT-cells $CD3^+CD16^+CD56^+$: 0.11±0.08x109 cells/l and NK cells: 0.31±0.18x109 cells/l.

Absolute counts of cytotoxic T-cells, Tregs and B-cells decreased with age (p= 8.73x10-5; 3.73x10-2; 3.46x10-2) but by contrast NK count increased (p= 1.25x10-2). No correlation of the counts of neutrophils, eosinophils, B-lymphocytes, T-helper cells, $CD4^+CD8^+$ T-cells, typical and atypical monocytes with age was found.

NK, typical and atypical monocyte counts were significantly lower for females than for males (p =0.03; 1.73x10-3; 0.014), while women had higher counts for cytotoxic T-cells (p= 0.044), T-helper cells (p= 1.74x10-4), Tregs (p= 3.41x10-3), $CD4^+$ $CD8^+$ T-cells (p= 1.98x10-3) and B-cells cells (p= 2.23x10-4).

Conclusion: Evaluation of the biological variability in this LIFE cohort may help to better discriminate between health and disease status and therefore to develop fast strategies for early prognosis of disease. In comparison to recent studies publishing reference values the LIFE study benefits from an up to 10fold larger study group and it covers the grey zone between children and old adults, which have been less been focused on in other studies. A decrease in the count of cytotoxic T-cells and Tregs in the elderly may indicate a higher risk of infection than four young adults. Also women seem to have a higher ability to respond to infectious diseases than men.

Counting Cytokine-Producing T Helper Cells: Concentration of Calcium in the Medium Can be Critical

Jakob Zimmermann, Andreas Radbruch, Hyun-Dong Chang
German Rheumatism Research Centre (DRFZ) Berlin, Germany

Antigen-experienced T helper (Th) cells can be classified into several lineages like Th1, Th17 etc. Depending on the adopted phenotype, Th cells express a specific master transcription factor and a hallmark cytokine like ROR-γt and IL-17A for Th17 cells. To define the phenotype of Th cells in an experimental setting, many scientists rely on the intracellular staining of hallmark cytokines. We found that, when using cytokine staining with standard RPMI medium for PMA/Ionomycin restimulation, the number of Th17 cells in a mouse model of colitis was dramatically underestimated as related to cells staining for ROR-γt. Interestingly, when we changed the medium during the period of restimulation to IMDM, the numbers of IL-17$^+$ Th cells increased substantially and matched the frequency of ROR-γt$^+$ cells. Using IMDM did not only influence IL-17A levels, but also elevated the production of IFNγ, TNF, IL-10 and IL-22. Comparison of IMDM and RPMI recipes revealed a more than threefold higher concentration of calcium in IMDM vs. RPMI. Strikingly, when we supplemented RPMI with $CaCl_2$, we could recover the frequency of cytokine-producing cells revealed by IMDM. Our data show that the calcium concentration of the medium used for PMA/Iono restimulation of Th cells can be critical to reveal their full cytokine-producing potential. RPMI should not be used.

Modulation of T Lymphocyte Calcium Influx Patterns via the Inhibition of Kv1.3 and IKCa1 Potassium Channels in Autoimmune Disorders

Gergely Toldi
First Dept of Pediatrics, Semmelweis University, Budapest, Hungary

Introduction : The transient increase of the cytoplasmic free calcium level is a key signal transduction mechanism in the process of lymphocyte activation. Voltage-sensitive Kv1.3 and calcium-dependent IKCa1 lymphocyte potassium channels have been implicated as important targets of selective immunomodulation in autoimmune disorders. The relationship between the influx of calcium through the cell membrane and the efflux of potassium makes the activation and cytokine production of T lymphocytes sensitive to pharmacological inhibition of Kv1.3 and IKCa1 channels. We aimed to characterize the effects of lymphocyte potassium channel inhibition on peripheral blood T lymphocyte activation in a number of immune-related disorders, such as rheumatoid arthritis, multiple sclerosis, type I diabetes and stroke induced immunosuppression compared to healthy individuals.
Methods : We evaluated calcium influx kinetics following PHA activation in CD4, Th1, Th2 and CD8 cells. We also assessed the sensitivity of the above subsets to specific inhibition of the Kv1.3 and IKCa1 potassium channels. Cells were stained with cell surface markers and intracellular calcium binding dyes (Fluo-3 and Fura Red) and flow cytometry analysis was performed in a kinetic manner (BD FACSAria). Data acquired from the measurements were evaluated using a novel algorithm based on the calculation of a double-logistic function for each recording (www.facskin.com). Specific parameter values describing each function were used to compare individual measurements in an objective manner.
Results : The time when the peak of calcium influx in T lymphocytes was reached decreased in autoimmune patients compared to healthy individuals, indicating that these cells are in a state of sustained reactivity due to the ongoing autoimmune reaction. In healthy controls the inhibition of the IKCa1 channel decreased calcium influx in Th2 and CD4 cells to a lower extent than in Th1 and CD8 cells. On the contrary, the inhibition of Kv1.3 channels resulted in a larger decrease of calcium entry in Th2 and CD4 than in Th1 and CD8 cells. In the investigated autoimmune patients a greater decrease of calcium influx upon the inhibition of the Kv1.3 channel than that of the IKCa1 channel was observed in Th1 cells. However, the selectivity of the investigated inhibitors was limited in our experiments. The inhibitory effect was present not only in disease-associated CD8 and Th1 cells, but also in the anti-inflammatory Th2 subset. The induced decrease in their function could lead to unwanted side-effects and in a setback of therapy *in vivo*.
Conclusions : Based on our results, a number of dominant features of T lymphocyte calcium influx and its sensitivity to the inhibition of potassium channels were identified that were present in the investigated autoimmune diseases. Further studies are needed on human samples and experimental models to judge the usefulness of this approach in the fight against autoreactive lymphocyte subsets and harmful cellular responses in autoimmune patients.

Fluorochrome-Based Definition of Foxp3+ Regulatory T Cell Subphenotypes with Specialized Suppressor Functions

Karsten Kretschmer[1,2]
[1]Molecular and Cellular Immunology/Immune Regulation, DFG-Center for Regenerative Therapies Dresden (CRTD); [2]Paul-Langerhans Institute Dresden (PLID), Deutsches Zentrum für Diabetesforschung e. V. (DZD); TU Dresden, Germany

In mice and humans, $CD4^+$ regulatory T (Treg) cells expressing the forkhead box transcription factor Foxp3 play an essential role in the maintenance of immune homeostasis by regulating inflammatory responses against invading pathogens as well as preventing destructive autoimmunity. Both intra- and extrathymic T cell developmental pathways have been proposed to feed into the overall pool of mature $Foxp3^+$ Treg cells residing in peripheral lymphoid tissues. However, under physiological conditions, studies on the biology of intra- and extrathymically induced $Foxp3^+$ Treg cells have been hampered by the lack of unambiguous markers to discriminate such developmental Treg cell sublineages. Here, we report on double-transgenic mice, in which red fluorescent protein (RFP) is expressed in all $Foxp3^+$ Treg cells, whereas Foxp3-dependent green fluorescent protein (GFP) expression is exclusively confined to intrathymically induced $Foxp3^+$ Treg cells. This novel molecular genetic tool provides a unique opportunity to faithfully track and characterize naturally induced Treg cells of intrathymic (RFP^+GFP^+) and peripheral (RFP^+GFP^-) origin in otherwise nonmanipulated mice. The results show that extrathymic Treg cell induction substantially contributes to the peripheral pool of mature $Foxp3^+$ Treg cells in the steady state. Additionally, we provide direct evidence that intrathymically and peripherally induced $Foxp3^+$ Treg cells represent distinct phenotypic and functional sublineages. Notably, $Foxp3^+$ Treg cells of extrathymic origin appear particularly suitable to interfere with the autoimmune destruction of pancreatic beta cells in mouse models of human type 1 diabetes.

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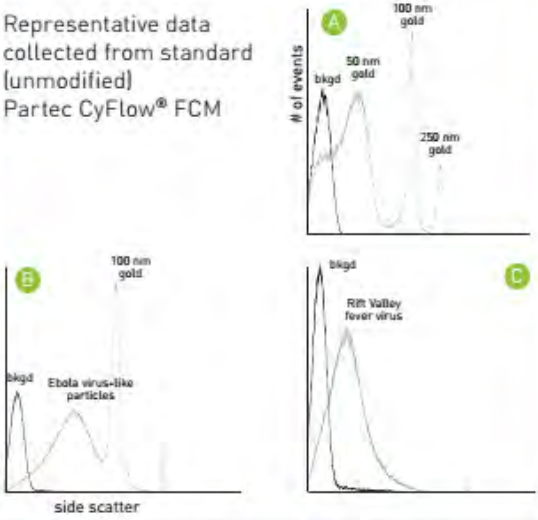

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Figure A 488 nm side scatter of a mixture of various size gold particles, ranging from 50 nm to 250 nm.

Figure B 488 nm side scatter of a mixture of Ebola virus -like particles (50-70 nm by 1-2 µm) and 100 nm gold particles.

Figure C 488nm side scatter of Rift Valley Fever virus (90-110 nm).

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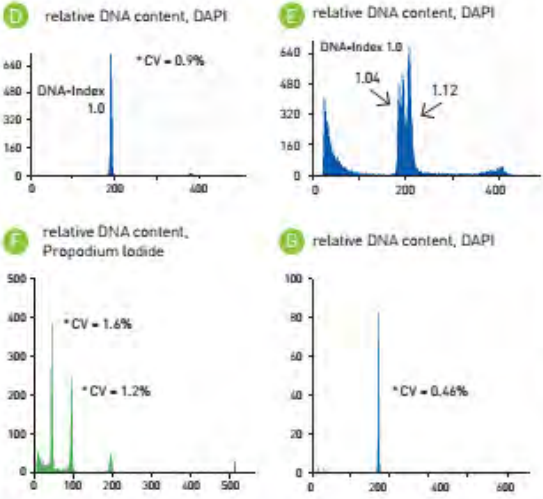

High Resolution DNA Analysis

Figure D Human lymphocytes stained with DAPI and analysed with Partec CyFlow® using UV Excitation

Figure E Human lymphocytes mixed with cells of a tumor biopsy, stained with DAPI and analysed with Partec CyFlow®

Figure F Leaf tissue of *Arabidopsis thaliana* stained with PI and analysed with Partec CyFlow®

Figure G Highest Sensitivity DNA Analysis of human lymphocytes using Partec CyFlow® with 365 nm UV LED excitation

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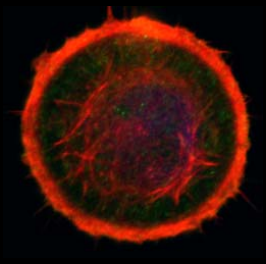
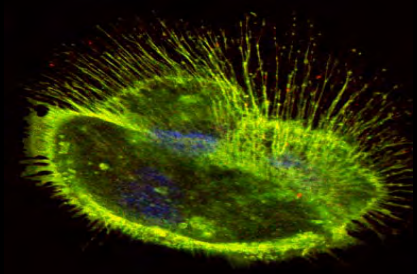


POSTER ABSTRACTS



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P1 – P29

Poster Session I:
Thursday - 10/Oct/2013, 11:40 – 12:25 am
Even numbers

Poster Session II:
Thursday - 10/Oct/2013, 12:30 – 01:15 pm
Odd numbers

P1 Prominin-1 and the Architecture of Microvillus and Primary Cilium

Kristina Thamm[1], Deimanté Šimaitė[1], Michaela Wilsch-Bräuninger[2], Wieland B. Huttner[2], Denis Corbeil[1]
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The apical domain of polarized epithelial cells is divided into planar and protruding regions that contain distinct subtypes of ganglioside-based membrane microdomains. By studying the subcellular localization of prominin-1 (CD133) we demonstrated previously that microvilli are enriched in ganglioside GM1 whereas the planar subdomain in GM3. In contrast, primary cilium contains both GM1 and GM3. Prominin-1 is a pentaspan membrane glycoprotein selectively associated with plasma membrane protrusions, and more than twenty alternative splice variants were described. Two of them (named s1 and s2) differ by the insertion of a facultative exon encoding for nine amino acids (PETVILGLK) within the first extracellular domain of the s2 variant. Although the relevance of these residues is unknown, their presence might modulate and/or interfere with putative interaction(s) of prominin-1 with ganglioside-based membrane microdomains. A potential ganglioside-binding site is found upstream this extra exon. Such protein-lipid interaction may determine the composition and/or organize the structure of cellular protrusions such as microvillus and primary cilium. Here, we investigated these issues using wild type or mutant forms of human prominin-1 and Madin-Darby canine kidney cells as a model of epithelial cells. Mutations in both splice variants (referred to as s1_2M or s2_2M) were created within the ganglioside-binding site. Confocal microscopy analysis revealed that cells over-expressing prominin-1 harbor a longer primary cilium compared to the ones found in untransfected cells. The effect is particularly striking with the s2 variant, although s1 also slightly increases the length of this sensory organelle. Remarkably, the expression of the mutated prominin-1.s2_2M showed that this effect is dependent of an intact ganglioside-binding site. Next, we examined by scanning electron microscopy the effect of prominin-1 on the architecture of microvilli. The over-expression of both splice variants does not lead to an obvious increase in the number (or density) of microvilli, however, we could observe that the s1, but not s2 variant, often created branched microvilli. Surprisingly, the expression of the prominin-1.s1_2M resulted in microvilli with a globular or knob-like structure at their tip. These results highlight potential interactions of prominin-1.s1 and s2 with gangliosides within the microvillar and primary ciliary membrane, respectively. Collectively, our data suggest that prominin-1 may play somehow a scaffolding role in the organization of plasma membrane protrusions, and its unique membrane topology, in particular the two large glycosylated extracellular loops, together with the conical shape of GM1 may promote and/or stabilize positive membrane curvature.

P2 Test of Red Blood Cell Agglutination Induced by Lectins for Diagnosis of Myocardial Infarction

Jozsef Bocsi[1], Kathleen Nieschke[1], Anja Mittag[1], Thomas Reichert[2], Wiebke Laffers[3], Arkadiusz Pierzchalski[1], Joachim Piltz[4], Hans-Jürgen Esche[4], Günther Wolf[2], Ingo Dähnert[1], Attila Tarnok[1]
[1]University of Leipzig; [2]GEMAC, Chemnitz; [3]University of Bonn; [4]amtec, Leipzig, Germany

Myocardial infarction (MI) is an acute life-threatening disease with a high incidence worldwide. Aim of this study was to test lectin-carbohydrate binding-induced red blood cell (RBC) agglutination as an innovative tool for fast and precise diagnosis of MI.

Five lectins (Ricinus communis agglutinin (RCA), Phaseolus vulgaris erythroagglutinin (PHA), Datura stramonium agglutinin (DSA), Artocarpus agglutinin (ArA), Triticum agglutinin (TA)) were tested for agglutination characteristics in patients with MI or angina pectoris without MI (AP) and healthy volunteers (HV) as control. RBC agglutination was analyzed by light absorbance of a stirred RBC suspension in a broad range in green and red light in an agglutimeter (amtec) for 15 min after lectin addition. Mean cell count in aggregates was estimated from light absorbance by a mathematical model.

Each lectin induced RBC agglutination. RCA led to the strongest RBC agglutination (~150 RBCs/aggregate), while the others induced substantially slower agglutination and lead to smaller aggregate sizes (5-20 RBCs/aggregate). Lectin-induced RBC agglutination of patients with MI or AP was generally higher than for HV. However, only PHA-induced agglutination clearly distinguished MI from HV. AP agglutination was intermediate (MI>AP>HV)

It is possible to differentiate between patients with MI and HP based on PHA-induced RBC-agglutination. We hypothesize that pathological changes induce modification of the carbohydrate composition on the RBC membrane and thus modify RBC agglutination. Occurrence and distribution of carbohydrates on RBC membranes provide evidence about MI. This novel assay could serve as a rapid, cost effective valuable new tool for diagnosis of MI.

Acknowledgement: This work was supported by Sächsische Aufbaubank (SAB No: 11624/1845) and BMBF Project MaDaKos (BMBF No.: 990101-088.12/2010-11/2013).

P3 Characterization of Radioresistant Prostate Cancer Populations

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Medical Faculty Carl Gustav Carus, Dresden University of Technology, Germany

Prostate cancer is the second leading cause of cancer-related deaths worldwide. Radiotherapy is one of the key treatments for prostate cancer. Although a significant proportion of men are diagnosed with curable localized prostate cancer, the radioresistance of prostate tumor cells is a practical limitation of radiotherapy, especially in advanced-staged and relapsed tumors. Resistance to radiation therapy has been reported for a small cell population called cancer stem cells (CSCs) or tumor progenitors in various tumor types, which are responsible for tumor formation, therapy resistance and metastasis. Moreover, CSC marker expression is correlating with poor clinical outcome. We found that ionizing radiation treatment enriches for the CSC phenotype and properties by preferential survival and expansion of tumor progenitor cells *in vitro*, *in vivo* and in *ex vivo* irradiated primary prostate cancer samples in a time- and dose-dependent manner. Our results indicate that $ALDH^+$ progenitor cells are less sensitive to ionizing radiation than $ALDH^-$ cells and show enhanced DNA double strand break repair, activated cell survival and self-renewal and induced epithelial-mesenchymal transition signature. We found that X-ray irradiation induces and enriches bulk tumor cells to a more clonogenic and radioresistant population. Moreover, these radioresistant tumor progenitors undergoes a phenotypically and functional switching during the course of irradiation. Global gene expression analysis revealed that the Wnt/β-catenin signaling pathway in combination with epigenetic changes are the main causative factor involved in the induced changes of CSCs during the course of irradiation. The combination of irradiation with therapies directed against CSCs, including therapeutically inhibition of ALDH activity and Wnt signaling leads to significant increase of prostate cancer radiosensitivity *in vitro*. These results suggesting that controlling the properties of CSCs during radiation therapy is ultimative for the optimization of treatment strategies. Furthermore, CSC markers are beneficial in the prediction of tumor radiocurability, and combination of irradiation with therapies directed against CSCs can be a useful strategy to improve cancer treatment.

P4 *In Vivo* Environment Determines CD44 and CD133 Surface Expression and Engraftment of LS1034 Colorectal Cancer Cells

Thiemo F. Dinger, Philipp Grosse-Gehling, Claudia Dittfeld, Leoni A. Kunz-Schughart
OncoRay - National Center for Radiation Research in Oncology, TU Dresden, Germany

Background & Aim

The search for theranostic biomarkers to identify putative CSC populations in solid cancers is of particular interest in translational research to develop patient-individualized treatment strategies. Surface proteins under investigation in colorectal cancer (CRC) are CD133 and CD44. In previous studies using establishes CRC cell lines, we found neither CD133 nor CD44 surface expression *in vitro* to define engraftment. In contrast, we observed a redistribution of cell subpopulations in xenografts derived from cell fractions sorted according to these markers. LS1034 cells which appeared to be negative for CD44 *in vitro* even showed induction of CD44 *in vivo*. We therefore aimed to elucidate the impact of *in vivo* CD44 presentation in this CRC model on tumorigenicity and to locate CD44-expressing cells within the tumor mass.

Materials & Methods

CD133/CD44 antigen pattern was studied by flow cytometry and Western blotting. Xenograft formation was monitored after s.c. injection of 10 – 10,000 LS1034 cells into the hind limbs of NMRI(nu/nu) mice with Matrigel. $CD133^+$ and $CD133^-$ subpopulations were separated by fluorescence-activated cell sorting (FACS) and analyzed for their tumorigenic potential. Marker expression in the resulting tumors was determined. Selected primary xenografts were dissociated, stained for HLA, CD44, CD133, and membrane integrity (PI-), sorted by FACS and re-injected for secondary engraftment. Another aliquot was subjected to frozen section immunofluorescence staining.

Results & Conclusion

The *in vitro* cell surface presentation of CD133 did not correlate with tumorigenicity in LS1034 cells. However, the $CD44^+/CD133^+$ cancer cell fraction in LS1034 xenografts showed highest secondary engraftment, while $CD44^-/CD133^+$ cells were intermediate and the $CD44^-/CD133^-$ tumor cell fraction did not produce secondary tumors. Interestingly, tumorigenic $CD44^+$ LS1034 cells are primarily located in perivascular but not hypoxic areas. We conclude that the expression pattern of the markers and the behavior of the cells are critically affected by the *in vivo* microenvironment. Ongoing studies focus on the characterization of CD44 (variants) and microenvironmental constraints determining the *in vivo* expression profile.

This work was supported by DFG grant KU 971/7-1.

P5 Focused and Homogenous Application of 20 MeV Protons Induce Differences in γ-H2AX Loss Kinetics

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[1]Klinikum Rechts der Isar, Technische Universität München; [2]Universität der Bundeswehr München, Neubiberg; [3]Department of Pediatric Cardiology, University of Leipzig, Germany

Background and Aims: Due to their physical and radiobiological properties heavy ions are of special interest for tumor therapy. The aim is to simulate the increased RBE of heavy ions using spot application of a bunch of protons.

Materials and Methods: Counted protons were applied either in a focused mode in a 5x5 μm^2 matrix with 100 protons per point or a nearly homogenous mode at the same dose of 1.7 Gy. CENP-F expression was measured for separation of cells in G2 and S-phase using Laser-Scanning-Cytometry. The decay of the integrated fluorescence of γ-H2AX was measured as a marker for double strand breaks. Fluorescence signals from cells irradiated 15 minutes before fixation were used for intensity calibration, which minimizes errors caused by variation between different dishes.

Results: Our findings indicate significant differences in the cell cycle between both application modes in HeLa cells. After 10 hours cell cycle arrest in G2 phase was about 10% higher ($p=0.02$) in the 5x5 µm group than in the homogenous mode. Measurements of the γ-H2AX-signalling from 15 min to 10 hours resulted in a 52% decrease for the 5x5 µm group and in a 62% decrease ($p=0.01$) for the homogenous group. The time-effect relationship was fitted by a bi-exponential function showing a fast and a slow component with identical half-life values.

Discussion and Conclusions: DSB repair in mammalian cells proceeds through two genetically separable pathways, homologous recombination (HR, slow) and non-homologous end-joining (NHEJ, fast). Our data supports the assumption that exposure of cells to a bunch of highly focused protons induces a higher amount of complex or severe DNA damage, like it is also found for high-LET irradiation, which requires HR rather than NHEJ for repair. These findings represent an important step towards a better understanding of the increased RBE of heavy ion irradiation.

Supported by the DFG Cluster of Excellence: Munich-Centre for Advanced Photonics.

P6 The Tumor Pathophysiological Milieu Affects Presentation of Putative Cancer Stem Cell Biomarkers

Philipp Grosse-Gehling[1], Thiemo F. Dinger[1], Constantin Mamat[2], Leoni A. Kunz-Schughart[1]
[1]OncoRay - National Center for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, TU Dresden; [2]Radiopharmaceutical & Chemical Biology, Helmholtz-Zentrum Dresden-Rossendorf, Germany

Background

The cancer stem cell (CSC) hypothesis states that only a fraction of cells within a tumor – the CSCs – possess multipotency and unlimited proliferative potential. A variety of biomolecules is discussed as potential markers to enrich or even isolate CSCs. In colorectal cancer (CRC) the most widely used biomarkers in this context are the transmembrane proteins CD44 and CD133 as well as enzymes of the aldehyde dehydrogenase superfamily. However, it is still unknown if the expression and cellular presentation of these biomarkers is prone to changes in the microenvironment and tumor pathophysiological milieu conditions such as tissue acidosis, oxygen deficiency or accumulation of catabolites. This was studied in various CRC cell lines; data will be presented for a model of particular interest.

Materials & Methods

We exposed LS1034 cells in serum-supplemented medium to different conditions such as low pH value, high lactate burden or hypoxia, and 2-D exponentially growing cells were compared with both confluent and non-adherent 3-D growth conditions. Results were related to biomarker expression profiles obtained from xenografts as well as immunofluorescence analyses of tumor sections.

Results & Conclusion

The biomarker profile *in vivo* differed substantially from that observed *in vitro*, characterized by a massive increase in CD133 and ALDH signal. Also, a fraction of cells expressed CD44 *in vivo*, whereas the cells *in vitro* appeared to be negative for CD44 on the cell surface. Interestingly, an increased ALDH and CD133, but not CD44 expression could be induced by incubation of cells in low pH medium independent of the lactate load, indicating that under these conditions a CSC surface phenotype is developed. On the other hand, non-adherent, 3-dimensional growth induced CD133 expression after 4 days, which subsequently decreased again after 10 or 20 days, respectively, whereas induction of CD133 expression through confluent growth remained stable. However, CD44 was not expressed under any of the tested conditions. These results illustrate the strong impact of the microenvironment on biomarkers used for the identification of putative CSCs.

This work was supported by DFG grant KU 971/7-1.

P7 Arginine Deprivation versus Citrulline Supplementation: Impact on Radiosensitivity in Colorectal Cancer Cells

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Background & Aim: Arginine deprivation is currently undergoing clinical trial as a treatment for several tumor types with low or absent argininosuccinate synthase (ASS) expression. ASS is an essential enzyme in arginine synthesis from its precursor citrulline (Cit) which is present in patients' blood stream. We have already shown that arginine deprivation alone as well as in combination with the natural arginine analogue canavanine (Cav) enhances radiosensitivity of HT29 colorectal cancer cells. The aim of the ongoing project is to study the impact of Cit availability on cancer cell survival and sensitivity to irradiation thus better mimicking conditions *in vivo*.

Methods: Spheroids of two colorectal cancer cell lines (HCT116, HT29) were grown in liquid- overlay and analyzed via semi-automated diameter and volume measurements. Arginine-deprivation was achieved by specially formulated arginine-free medium.

Results: Cit in concentration equimolar to arginine in DMEM (0.4 mM) partly restored spheroid growth of both tested cell lines upon arginine deprivation. Addition of 0.1 mM Cav to arginine-free medium resulted in 3-D growth arrest even upon Cit supplementation. In parallel, detachment of cells from spheroids was observed already after 3 days of treatment. Growth abrogation in both cell models was accompanied by the accumulation of cleaved poly (ADP-ribose) polymerase, a typical hallmark of apoptosis. Arginine supplementation restored growth of both spheroid models even after 12 days of Cav treatment in arginine-free medium containing Cit. However, a cell-line specific lag phase and rate of spheroid re-growth was seen. Nevertheless, Cit supplementation reduced but did not abrogate the radiosensitizing effect induced by the combination of arginine deprivation and Cav treatment in HT29 spheroids.

Conclusion: Our results suggest that arginine-deprivation combined with arginine analogue(s) could be a promising strategy for increasing the curative potential of radiotherapy regardless of the tumors ability to utilize Cit.

This work was supported by the DAAD.

P8 Development of a Novel ELISA for Detecting Inducible Hsp70 in the Serum

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The members of the heat shock protein 70 family are known to play a major role in assisting protein folding, preventing protein aggregation and transport of proteins across membranes. The heat shock cognate protein 70 (Hsc70) that is constitutively expressed in the cytosol of eukaryotic cells is only moderately up-regulated following stress. The major stress-inducible heat shock protein 70 (Hsp70) which is found at low levels under physiological conditions is highly up-regulated after various stress conditions, including heat, oxidative stress and/or chemo- and radiotherapy.

Tumor cells, in contrast to normal cells, frequently express higher basal levels of inducible Hsp70 in the cytosol and express it on their plasma membrane. In accordance with that, elevated levels of Hsp70 have been detected in the supernatant of tumor cell lines and also in the blood of cancer patients.

We developed a novel sandwich ELISA for the detection of inducible Hsp70 in the serum. Capture of the protein is achieved with a polyclonal Hsp70 antiserum and detected by using the biotinylated monoclonal anti-Hsp70 antibody. This antibody binds specifically to Hsp70 and does not cross-react with the highly homologous constitutively expressed Hsc70. After incubation with horseradish peroxidase-conjugated streptavidin, antibody binding is quantified by measuring substrate conversion in an ELISA microplate reader.

This ELISA will be validated in upcoming experiments and used for the screening of cancer patients to monitor their Hsp70 serum levels before, during and after therapy. By taking into account tumor entity, stage, therapy and clinical outcome the ELISA will be tested as a novel prognostic tool.

P9 Arginine Deprivation as a New Strategy for Brain Cancer Therapy?

C. Noreen Hinrichs[1], Mirjam Ingargiola[1], Achim Temme[2], Barbara Klink[3], Leoni A. Kunz-Schughart[1]

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Introduction: Arginine is a highly versatile semi-essential amino acid necessary for numerous intra- and extracellular processes. Previous data revealed that tumour cells can show lack or reduction of the key enzyme in arginine synthesis, argininosuccinate synthetase (ASS), making them dependent on external arginine supply. Arginine deprivation therapy (ADT) is a strategy to induce a cytostatic effect in tumour cells and has already entered clinical trial for various cancers. The treatment represents a systematic approach and its efficacy is supposed to be independent of any local penetration and diffusion limitations including the blood-brain barrier. We recently described that the starvation for arginine results in radiosensitization in colorectal cancer cells *in vitro*, and thus hypothesized that ADT plus irradiation could also be a promising treatment strategy for glioblastoma which are intrinsically highly resistant to external irradiation.

Methods: The impact of ADT with or without supplementation of the arginine precursor citrulline on different glioblastoma cell lines (U251-MG, U138-MG, U87-MG, U87-MG-shp53) was monitored *in vitro* in growth and colony-forming cell assays, and the cell cycle distribution was analyzed in static flow cytometric DNA histograms. Furthermore, the efficacy of combined ADT and radiotherapy versus radiotherapy alone was assessed.

Results: All glioblastoma cell lines cultured in arginine-free medium or in the presence of an arginine-degrading enzyme showed growth inhibition and a decreasing survival fraction with increasing ADT interval. Combined treatment of ADT and irradiation caused a significant radiosensitization in the ASS low expressing U251-MG, but not in U87-MG and U87-MG-shp53 cells characterised by higher ASS protein levels. Cell cycle analysis confirmed that radiosensitization does not result from ADT-induced cell cycle arrest in the radiosensitive G2/M-phase. Citrulline supplementation did not compensate for the ADT induced growth inhibition and radiosensitization.

Conclusion: ADT appears to be a promising strategy to treat glioblastomas. For one glioblastoma cell line a combination of ADT and irradiation seems to be particularly potential. The relevance of ASS level as a theranostic biomarker for ADT has to be further investigated.

This work was supported by the Else Kröner-Fresenius Foundation.

P10 Stimulation of Antibody Dependent Cellular Cytotoxicity (ADCC) upon Failure of Cellular Tumor Suppression of Breast Cancer Cells by Trastuzumab

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Introduction: Therapy of Her2-positive breast cancer patients using Trastuzumab can prolong the recurrence free and overall survival in both metastatic and the (neo-)adjuvant setting. However, about 40-60 % of all patients acquire cellular resistance during therapy. The therapeutic efficiency of Trastuzumab treatment is not only due to inhibition of tumor cell growth e. g., by inhibiting Her2 signaling but moreover can cause tumor cell eradication by triggering the immune system. Hence, aim of this study was to evaluate the interaction of NK- (effector) and tumor- (target) cells initiated by Trastuzumab and to evaluate strategies to enhance NK-cell mediated tumor cell defense in Trastuzumab sensitive and insensitive tumor cell lines.
Methods: In order to generate cellular resistance the Her2-positive Trastuzumab-sensitive breast cancer cell line BT474 was continuously exposed to 10 µg/ml Trastuzumab for a period of four months. The proliferative capacity was repeatedly analyzed by flow cytometry. Simultaneously binding efficiency of Trastuzumab was evaluated. The immunologically mediated cytotoxicity was investigated in cocultures of NK- and Trastuzumab sensitive vs. resistant BT474 cells. Effector and target cells were analyzed by multiparametric phenoytping (NK cell activation e.g., Perforin-, IFNγ release) and Annexin-Dapi assays (tumor cell killing). In addition the kinetics of Trastuzumab treatment efficiency in terms of target cell number and viability was monitored by using the xCELLigence technique.
Results: The originally highly sensitive BT474 cells acquired resistance to Trastuzumab after prolonged treatment (four months). The proliferative fraction was 25.3% ± 2.4% in sensitive wildtype (WT) cells vs. 27.7 ± 0.27% in resistant cells. Despite resistance the BT474 cells maintained the original Her2 expression level and binding capacity of Trastuzumab was unaltered (WT: 0.496 ± 0.06 vs. Res: 0.54 ± 0.07).

$$\frac{\textit{Trastuzumab - Bindung}}{\textit{Her2 - Expression}}$$

Despite of cellular resistance BT474 remained susceptible to immunological anti-tumor effects: NK-cell mediated antibody-dependent cellular cytotoxicity (ADCC) was 173% ± 8.4% in WT-BT474 and 159% ± 6.9% in resistant cells after 48h of coculture. xCelligence kinetics revealed a gradually increasing tumor cell killing in coculture over the course of 96 hrs. Tumor cell killing is due to an antibody mediated colocalisation of NK- and tumor cells that triggers Perforin release. ADCC in resistant BT474 cells can be moderately enhanced by IL-15 stimulation [10 ng/ml] of NK cells and to a greater extent by an increased effector target cell ratio.
Conclusion
Multiparametric phenotyping by flow cytometry enables the phenotypic and functional characterization of effector (NK) and target (tumor) cells derived from cocultures. In this experimental setup Trastuzumab triggers ADCC both in Trastuzumab sensitive and resistant cells. ADCC efficiency can be enhanced by IL-15 stimulation of NK cells, a promising approach that can potentially be extended to other antibody therapy regimens.

P11 Antigen-Reactive T Cell Enrichment for Direct, High Resolution Analysis of the Human Naïve, Memory and Regulatory CD4+ T Cell Repertoire

Petra Bacher[1], Anne Schönnbrunn[2], Janka Teutschbein[3], Olaf Kniemeyer[3], Mario Assenmacher[4], Axel A. Brakhage[3], Andreas Thiel[2], Alexander Scheffold[1,5]

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Antigen-specific CD4+ T cells play a central role in the immune defense against pathogens or tumors but also in immunopathology, such as autoimmunity or allergy. Due to technical limitations, comprehensive information about the frequency and phenotype of rare antigen-specific CD4+ T cells against many disease-relevant antigens is missing. In particular the nature of the human naive as well as regulatory T cell (Treg) repertoire is elusive. The converse expression pattern of the activation markers CD154 (CD40L) and CD137 (4-1BB) has recently been demonstrated to allow the direct detection of rare antigen-specific conventional T cells and Treg from peripheral blood (Schönnbrunn et al. 2013 J Immunol.). We have developed a new, sensitive detection system for antigen-specific T helper cells, based on a combination of magnetic pre-enrichment and multi-parametric flow-cytometric analysis of CD154 and CD137 expressing CD4+ T cells directly from human peripheral blood. The high sensitivity of 1 cell out of 10^5 to 10^6 enables the direct enumeration of conventional memory and naïve CD4+ T cells, as well as regulatory T cells reactive against basically any recall or primary antigen. The magnetic enrichment of few antigen-reactive T cells from large starting cell numbers (10^7 to 10^8) allows to rapidly analyze sufficient numbers of target cells to discriminate even small functional or phenotypic subpopulations within the total antigen-specific T cell pool, undetectable by conventional methods. We used this assay for a first characterization of tumor-, auto- and neo-antigen reactive CD4+ T cells from healthy subjects as well as for the in depth characterization of rare pathogen specific T cells. Importantly, the high sensitivity allowed us to show, that naive but also memory T cells specific for various tumor-, auto-, or neoantigens, are present even in healthy subjects. The technology enabled further to identify a highly diverse functional repertoire within the conventional CD4+ T cell response against various pathogens, as well as a largely expanded Treg population specific for mucosal fungi as part of the physiological human T cell repertoire.

P12 A new Method for Detection of Membrane Bound Glucocorticoid Receptors: Comparison of the Liposome Procedure and the FASER Technique

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Background: Flow cytometry is a widely-used and powerful tool for the characterization of cells according to their expression of specific proteins. Since the sensitivity of this method is still limited there is a need to develop new techniques in order to identify molecules which are expressed in very low but functionally relevant amounts.
In the past, we have successfully used a liposome-based high-sensitivity immunofluorescence technique to measure the expression of low abundant membrane bound glucocorticoid receptors on different cell types. Membrane bound glucocorticoid receptors (mGR) play a pivotal role in pathogenesis of chronic inflammatory diseases as indicated by clinical observations. Patients with Systemic Lupus Erythematodes (SLE) show high frequencies of mGR positive monocytes, sometimes even higher than found in patients with active Rheumatoid Arthritis. With increasing glucocorticoid dosages expression of mGR on monocytes of SLE-patients is downregulated, suggesting a negative feedback loop to control glucocorticoid action. These receptors represent an effective target for diagnosis and monitoring of different inflammatory diseases, but a feasible detection method is still necessary.
Objectives: We compare two methods of high-sensitive immunofluorescence staining – the well established liposome procedure with the commercialized FASER-technique.
Methods: HEK293T cells were cultured for 24h with/without 5µg/ml brefeldinA in a humidified incubator at 37°C. Human CD4 positive T-cells and CD14 positive monocytes were isolated via magnetic-activated cell sorting and subsequently cultured in RPMI 1640. Monocytes were incubated for 24h with/without 2 µg/ml LPS. For liposome based high-sensitivity immunofluorescence staining cells were incubated with the monoclonal (digoxigenin conjugated) anti-GR antibody, followed by incubation with anti-digoxigenin/anti-Biotin matrix. Subsequently biotinylated Cy5 liposomes were added. FASER technique was performed as described by the manufacturer (Miltenyi Biotec). Dead cells were excluded by adding PI before cell accquisition, using a BD FACS Calibur flow cytometer. The accquired data were analyzed using FlowJo 7.6.1 software.
Results: The human mGR, which cannot be reliably detected with conventional staining methods is detectable with the liposome procedure as well as with the commercialized FASER-APC technique. Furthermore, the FASER-APC-procedure is more sensitive (94,51% vs. 73,2%) and more specific (99,57% vs. 98,93%) compared to the liposome technique. Additionally, minor changes of mGR expression can also be demonstrated with the FASER technique. The FASER procedure shows technical advantages: the commercially available FASER-APC-kit is performed according to a standarized protocol and is less time consuming compared to the liposome procedure.
Conclusion & Perspectives: The human mGR is easily detectable with the commercialized FASER kits, which represent an alternative due to a consistent quality and a standardized production. This method facilitates the analysis of the role that mGR play in the pathogenesis of chronic inflammatory diseases and perhaps provoke new insights in glucocorticoid therapy.

P13 Development of Cytotoxicity Assay to Examine the Effects of Potential Drugs on Leukocyte Viability

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Introduction: Various advantageous characteristics provide particular chemicals (e.g. bioactive oligopeptides (OP)- a high potential for medical applications. In this context it is important to know the effect of potential drugs on primary human cells like peripheral blood leukocytes (PBL). In contrast to cell lines, actually used systems for first line drug screening, PBL are easier to provide and represents the reaction of human organism properly. We aimed to investigate toxic effects on PBL, developed very gentle and reproducible assay and used it to test toxicity of various OPs.

Material and Methods: 1. Assay development: Effects of various isolation methods (red blood cell (RBC) lysis; density gradient centrifugation a.o.) as well as different incubation tubes prepared from different plastics, anticoagulants and blood sources on PBL viability (incubated for 60 min on 36°C) were tested using propidium iodide-uptake by perished cells and flow cytometry.

2. OP effects: Toxic and time-depended OP-effects (10-60 min, 36 °C, 0-100 µg OP/ml) on human PBL cell subtypes were analyzed by immunophenotyping.

Results: 1. RBC lysis by hypotonic shock (dH_2O) led to highest viability (85%) as compared to NH4Cl-Lysis (49%). Density gradient centrifugation led to neutrophil loss and removed RBC not completely. In contrast to the other methods PBL-subpopulations were clearly identifiable using FSC-SSC light scatter. Heparin as anticoagulant yielded higher viability than EDTA. Use of conical micro-reaction tubes (polypropylene, PP) yielded highest viability (99%) in contrast to other test tubes (PP round-bottom tubes: 60%, PP: 62%, Polystyrene (PS): 64%; 2 ml micro-reaction tubes (round, PP): 64%). This method worked equally well with venous and capillary blood. Viability of PBL from both blood sources did not differ.

2. Subsequently, toxic effect of two OPs was analyzed. Hemolytic affecting OP 1 did not affect vitality of PBL, whereas hemolytic OP 2 decreased viability of PBL in a time- and dose depended manner.

Conclusion: Toxicity assay on primary cells is rare in the literature but essential to understand drug effects on the organism. To this end, a minimal harmful preparation technique for freshly drawn human PBL was developed and evaluated. It consists of dH_2O-lysis of erythrocytes from heparinized blood and incubation in conical polypropylene micro-reaction tubes. Only after developing this gentle assay we could observe that different OPs kill PBL selectively. This assay enables for large scale toxicological screening and pharmacological testing of OP variants on primary PBL.

P14 Cytometric Evaluation of the Efficiency of Antagomir Uptake by T and B Lymphocytes

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MicroRNAs are small non-coding RNAs which act to negatively regulate gene expression on the post-transcriptional level. Loss-of-function experiments are the most powerful tools for the characterization of the biological significance of a given microRNA, e.g. using chemically modified microRNA-complementary oligonucleotides like Antagomirs.

To unfold their inhibitory capacity, Antagomirs need to enter the cytoplasm of the target cell population. We have developed a quick and easy protocol for the knock-down of microRNAs in primary murine lymphocytes. On the example of an Antagomir directed against microRNA-148a we demonstrate the efficiency of antagomir uptake by image stream cytometry.

For later analysis or use of antagomir-treated cells in adoptive transfer experiments low cytotoxicity combined with high knock-down efficiency is required. We examined concentration-dependent knock-down efficiencies as well as cytotoxic effects of antagomir-148a in murine T and B lymphocytes by quantitative real-time PCR and flow cytometry.

Various high concentrations of non-targetting Antagomirs show no immediate effect on cytotoxicity whereas under the same incubation conditions an effective knock-down of microRNA-148a of more than 87% in B cells and more than 98% in T cells could be achieved. A knock-down efficiency of this magnitude generally allows for a reliable exploration of phenotypic effects in loss-of-function experiments.

P15 CHIC – an Automated Approach for Analyzing Microbial Community Dynamics

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Altering environmental conditions change structures of microbial communities. These effects have an impact on the single cell level and can be sensitively detected using community flow cytometry. However, although highly accurate, microbial monitoring campaigns are still rarely performed applying this technique. One reason is the limited access to pattern analysis approaches for the evaluation of microbial cytometric data. Here, we present a new analyzing tool, Cytometric Histogram Image Comparison (CHIC), which realizes trend interpretation of variations in microbial community structures i) without any previous definition of gates, by working ii) person independent and iii) with low computational demand. Various factors influencing a sensitive determination of changes in community structures were tested. The sensitivity of our technique was found to discriminate down to 0.5 % internal variation. The final protocol was exemplarily applied to a complex microbial community dataset and correlations to experimental variation were successfully shown.

P16 Cytometric Barcoding of Bacterial Populations in a Wastewater Treatment Plant

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Wastewater treatment plants suffer instabilities during the water treatment process when the microbial community is not stable enough to withstand changing inflow conditions. Therefore, the microbial community within the different stages of a wastewater treatment plant acts like a whole cell biosensor that allows monitoring of the process functionality.

A communal wastewater treatment plant was sampled for 29 days at the primary clarifier and both of the subsequent aeration tanks. Samples were analyzed flow cytometrically determining the DNA content of each individual cell. In addition to this, abiotic parameters were measured to assess the process functionality. From each cytometric dataset cell numbers of dominant subcommunities were determined and analyzed using cytometric barcoding.

The analysis showed that the stability within the microbial community differed between the sampling points, the primary clarifier being more unstable than the aeration tanks. It also showed how certain inflow conditions affected different subcommunities and therewith the whole wastewater purification process.

For microbial community analysis the here applied approach offers many advantages. It is fast and can be applied to every experiment were microbial populations are cytometrically measured. Additionally, the software needed is available as ready to use R-based macros.

P17 Subpopulation-Proteomics in Prokaryotic Populations

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Population heterogeneity plays an increasing role in biotechnological applications. Bulk analysis of process parameters such as optical density or biomass ignore the overwhelming variety of cells regarding morphology, physiology and production. Even microbial populations derived from one single clone are far from being homogeneous. Factors such as micro-environmental conditions, cell cycle stage, genetic inventory and plasmid copy number may cause cellular heterogeneity, and the resulting sub-populations can be recognized in high throughput using flow cytometry. Here, a novel combination of flow cytometric cell sorting and protein mass spectrometry was used to analyze sub-populations of different productivity. Cells of Pseudomonas putida KT2440 pCOM10-StyA::EGFP-StyB were identified and sorted regarding EGFP expression (yes/no) and inclusion body formation (yes/no). Three different subpopulations yielded over 750 proteins from all cellular compartments. However, only minor differences in metabolic pathways were found, but proteins involved in stress response as well as plasmid encoded markers were strongly upregulated in EGFP expressing cells. This result hints towards the highly unequal distribution of plasmid copies as a source for cell heterogeneity.

P18 An easy-to-use Protocol for Two-Parametric Cell Cycle Analysis of Plant Cell Suspension Cultures Applied to *Harpagophytum procumbens*

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Plant cell suspension cultures can serve as production system for pharmaceutical active secondary metabolites or proteins [1]. *In vitro* cultures enable a GMP-compliant production completely independent from the environment that strongly influences the secondary metabolite content and the quantitative phytochemical composition in herbs for example. However, these cultures consist of undifferentiated cells which can be very heterogeneous in terms of proliferation activity, secondary metabolite production, and extend of agglomeration [2, 3, 4].
The development and modelling of bioprocesses using plant cell suspension cultures require the quantification of heterogeneities like proliferation activity. One-parametric cell cycle analysis does not provide enough information about the proliferation activity in asynchronously growing cell suspension cultures and are unable to detect the amount of quiescent cells [5]. More information on the cell replication within plant cell suspensions is offered by bi-parametric cell cycle analysis. It can be performed using the incorporation of BrdU, a thymidine analogue, into the DNA of proliferating cells. BrdU can then be detected by fluorescence-labelled antibodies or by the Hoechst-BrdU-quenching using flow cytometry.
As plant cells in suspension cultures normally not occur as single cells, nuclei have to be isolated in order to analyse them by flow cytometry but isolated nuclei can be sensitive against chemical and mechanical stress. This makes the use of multi-step protocols that are needed for the detection of BrdU with antibodies difficult or infeasible.
Therefore, a simplified protocol was developed, that allows cell cycle analysis using the Hoechst-BrdU quenching and PI as counterstain that is easy to handle and without harsh conditions for the nuclei. This protocol was proved in a growth experiment with a cell suspension culture of *Harpagophytum procumbens* using different BrdU concentrations.
The protocol is based on the isolation of nuclei and the staining with Hoechst and PI directly within the isolation buffer that makes it easy to handle. No centrifugation steps are necessary which allows the use also of fragile nuclei and further, fresh material can be analysed without long delays between sampling and analysis. It is possible to follow more than one cell cycle with a single, continuous incubation of a *H. procumbens* cell suspension culture with 10 or 100 µM BrdU and quiescent cells could be detected during the cultivation. The application of the described protocol should be extendable to plant tissue cultures and parts of intact plants.

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P19 Evaluation of Cells Stress in Biotransformation

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For the synthesis of enantiomerically pure compound the chemical repertoire of whole cell catalysts is of enormous importance. However, most of the substrates to be converted are xenobiotic or even toxic and have to be applied in low concentrations. In this study stereoisomerically pure cyclic cis-b-hydroxy esters, being important chiral pharmaceutical intermediates (CPI) should be produced by whole cell biotransfomation performed by yeast. In order to improve the stereoisomeric purity a physiologic active substance (PAS) was added. Since it has been shown that stress proteins are involved in the dehydrogenase activity should additional stress be exerted by the PAS caffeine on the biocatalyst to perform the transformations in the stress in physiology of the cell.

Indeed, stereoselectivity was increased by the addition of caffeine. But it also showed that the addition of caffeine decreased the cell viability by up to 20%. Since caffeine may evoke apoptosis in stressed cells, this could lead to programmed cell death in the reaction mixture and thus be a reason for the higher stereoselectivity.

To evaluate the extendof potential caffeine-induced apoptosis, the *S. cerevisiae* cells were analyzed by FCM on "Reactive Oxygen Species" (ROS), which are an important indicator of apoptosis.

It could be demonstrated that cells which are exposed to the precursor, have slightly higher levels of ROS. The combination of the precursor with caffeine led to a significant increase in ROS formation. Consequently, the combination of ß-keto esters and caffeine induced an apoptotic behavior, while the addition of ß-keto ester or caffeine alone leads to cell death without signs of apoptosis. Counterstaining with Propidum iodide should give indications on the cell-vitality. Dead cells should show a uniform high fluorescence peak here. After 24 hours of treatment with caffeine and precursor, peaks were observed at a lower fluorescence intensity. The smaller DNA fluorescence is either due to the activation of endonucleases which fragment the DNA, or the condensation of the chromatin DNA. The latter is a typical indicator for another programmed cell death. This result supports the hypothesis that apoptosis is induced in the biotransformation under these conditions.

P20 Robotic Platform for the Automated Handling and Monitoring of Mircrofluidic Chips

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A smartphone-sized microfluidic chip platform was invented at Fraunhofer IWS for the *in vitro* cultivation of human tissue. The platform consists of an active microfluidic System with pumps, valves and channels molded from PDMS. To automate the handling and the monitoring of the chips we developed a robotic platform. Therefore the handling of the chips includes automated fluid handling like dosing of liquid media and samples into the chips, the control of the valves and micro pumps, temperature control and the storage of liquid samples of the cell culture. For the monitoring of the chip the robotic platform includes a small microscope, a high frequency camera module for micro-PIV measurement, a miniaturized module for high sensitive, spatially-resolved fluorescent measurement and a miniaturized module for non-invasive oxygen measurement. The platform is capable to maintain five parallel processed chips. In order to display the functionality of the platform we performed a toxicity test with RPTEC cells treated with DMSO as well as a diffusion test with fluorescein through an alginate membrane and a layer of CaCo2-cells.

P21 New Method to Analyze Cumulative BrdU Labeling Data

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The cumulative Bromodeoxyuridine (BrdU) labeling protocol is a standard method to measure the cell cycle length and the S-Phase duration [Nowakowski et al., 1989]. Here, we overcome protocol limitations with a new data analysis method. This opens the possibility to apply the protocol to a wider range of experimental setups.

The original method is only applicable in a limited number of cases where, firstly, all cells *divide asymmetrically* (implying a constant number of cells), secondly, all cells *proliferate at the same rate*. Moreover it depends on a "good" initial guess of the cell cycle parameters and lacks rigorous error estimation.

Here, we extend the standard method by providing a suitable fitting algorithm that overcomes the aforementioned limitations. We apply the new algorithm to a biological test case: ependymal cells of the spinal cord of the axolotl (*A. mexicanum*). Finally, we investigate how multiple sub-populations cycling at different cell cycle lengths would shape the BrdU data.

Reference: Nowakowski , Lewin & Miller, 1989. J Neurocytol. 18: 311-318.

P22 Influence of PVA Coated Nanoparticles on Survival and Functionality of Human Immune Cells

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Background: Nanotechnology has developed into a key technology of the 21st century. Over the recent years, the number of nanotechnical products has received an enormous boost. More and more efforts are being done to use this technology in human medicine for diagnostic and therapeutic purposes. Therefore, crucial questions concern the safety aspects. The focus of our work here was to identify possible effects of nanoparticles on human immune cell function.

Objective: We analysed clinical relevant interactions between PVA coated nanoparticles (spions) and human immune cells.

Methods: 100µl of whole blood obtained from patients with rheumatoid arthritis (RA) or healthy donors were incubated with 100µl serum free RPMI 1640. Functionalised spions were added at varying concentrations, and cells were incubated for 24h. After lysis of erythrocytes, cells were stained for apoptosis and necrosis using Annexin V and 7AAD, respectively. Samples were analysed by flow cytometry. As a second approach, PBMCs were isolated from blood samples of healthy donors and RA patients, and CD4 positive T cells were separated via MACS-Sort. T cells were incubated with/without PHA and/or with/without PVA spions at different concentrations. Activation (CD25 expression) of cells was analysed by flow cytometry. Functionality was determined via proliferation measurements of CFSE (carboxyfluorescein diacetatesuccinimidyl ester) labeled T cells after 72h under normoxic (5% CO_2 and 18% O_2) or hypoxic (5% CO_2 and <1% O_2) conditions by flow cytometry.

Results: Altogether, blood samples from 18 healthy donors and 19 patients suffering from RA were analysed for induction of apoptosis and necrosis in different cell types. The results on cell survival did not demonstrate any short-term general toxicity of PVA spions at concentrations less than 1000µg/ml on the several different blood cell subsets examined. Furthermore, T cells were isolated from 14 healthy donors and 9 RA patients for functional analysis. There is no influence of PVA spions on T cell activation and proliferation at concentrations less than 1000µg/ml.

Conclusion: PVA coated nanoparticles at concentrations up to 1000µg/ml (i) do not increase the frequencies of apoptotic or necrotic human immune and (ii) do not impair crucial functional activities of human T cells such as activation and proliferation.

P23 Platelet Alterations in Stored Pathogen-Inactivated Platelet Concentrates – a Study Using Flow Cytometry and Transmission Electron Microscopy

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Objective: The risk for bacterial contamination during manufacturing of platelet concentrates (PC) can be significantly reduced by pathogen inactivation (PI) providing an irreversible crosslink of nucleic acids. In addition, this procedure should allow a prolongation of storage time of PC beyond 5 days. The aim of our study was to evaluate viability and activation stage of platelets after pathogen inactivation using flow cytometry (FC), and transmission electron tomography (TEM).

Material and Methods: PC were prepared by apheresis from 6 healthy donors with a Trima-Accel cell separator. PI was carried out using the InterceptTM blood system (Cerus Europe BV). Samples for FC and TEM analyses were prepared before PI, after PI, and after 4 and 7 days of storage in platelet additive solution. FC measurements included the determination of absolute platelet numbers, the surface expression of CD62P and the binding of annexin-V to the plasma membrane reflecting platelet activation and apoptosis respectively. A recently published score system (1) was used for evaluation of TEM panorama images (consisting of 8 digital images) allowing to classify platelet activation and alteration at a single cell level. Separation and TEM analysis of apoptotic platelets was performed by separation of annexin-V positive platelets using the MACS system.

Results: The percentage of CD62P positive platelets was slightly reduced immediately after PI but increased significantly during storage reaching a factor of 2 after 4 days and a factor of 4 after 7 days of storage. In parallel, also the percentage of apoptotic platelets increased during storage – above all, dramatically on day 7. Apoptotic and necrotic platelets led also to a 30-40% reduction of absolute platelet counts on day 7. In contrast, the score profiles did not change significantly. The percentage of unaffected and activated platelets remained relatively constant over a period of 5 days while
the percentage of degenerative and necrotic platelets increased constantly with prolonged storage time. Most notably on day 7, an increased number of necrotic platelets occurred where some of them formed small aggregates. In TEM, apoptotic platelets could not be clearly distinguished from necrotic platelets but were visualized after MACS separation.

Conclusion: PI is able to reduce the risk of transfusion-transmitted infection in the recipient of a PC. Nevertheless, from our point of view, a prolonged storage period beyond 5 days cannot be recommended.

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P24 An UV-C LED Sheath Fluid Desinfection Module for Flow Cytometric Cell Sorting

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Standard protocols for performing a sterile sort are based on washing procedures by using ethanol or other sterilizing reagents and should be performed in a time-consuming daily routine. However, these reagents are expensive and some are toxic for cells if they are not complete washed out. Usually cell sorters are not in a sterile environment and therefore the risk of recontaminations exists. The ability to prepare a cell sorter for stable aseptic sorting is essential and one of the biggest challenges in flow cytometry.

We use a compact UV-C light emitting diodes (LED) module to sterilize the sheath fluid at the point of use. The module is connected to the sheath fluid tubing and installed close to the nozzle. The sheath fluid flows through the module and is irradiated. At a UV-C irradiation dose of 300 mJ/m², we were able to reduce the number of germs up to 3 log units.

The experiments were performed with a FACS Diva cell sorter. But due to their compactness and flexibility UV-C LED modules can easily be customized and implemented to other existing and new developed cell sorter to ensure sterile cell sorting.

P25 Diminished Adhesion to Laminin and Delayed Integrin Signalling of Human Adipose Stromal Cells after GMP-Compliant Culture in Human AB Serum

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The clinical application of mesenchymal stromal cells (MSC) requires a production process compliant with Good Manufacturing Practice (GMP) guidelines. Pooled blood group AB human serum (HS) has been used to replace fetal bovine serum (FBS), critically rated by the regulatory agencies, supporting the expansion of adipose tissue-derived mesenchymal stromal cells (ASC). Comparing HS- and FBS-ASC revealed differentially expressed adhesion and extracellular matrix-associated molecules raising the question whether the serum source differentially affects adhesion of ASC.

Because previous microarray studies revealed a significant reduced expression of integrin a6 (CD49f) in HS-ASC, we compared ASC cultivated in FBS and HS with respect to expression of integrin subsets forming the laminin receptor as well as integrin signalling upon adhesion to laminin.

Consistent with a lower CD49f expression intensity of HS-ASC, adhesion to laminin was significantly reduced compared to FBS-ASC. Blockage of CD29 (integrin a6) and CD49f, but not of CD104 (integrin a4), reduced the adhesion to laminin, pointing at integrin a6/ß1 (VLA-6) as functional laminin receptor on ASC. Upon adhesion to laminin, very rapid changes of the cell shape occurred, characterized by lammelipodia formation. Downstream signal transducers of integrin, p-FAK and p-PAX were expressed predominantly at these focal adhesion sites and indicated a delayed integrin signalling of HS-ASC compared to FBS-ASC.

Our data indicate that varying the serum supplement may alter clinical application-relevant characteristics of ASC like adhesion to extracellular matrix molecules. The reduced interaction with i.e. laminin may cause reduced homing and engraftment. Thus because major changes in the manufacturing process such as changing the source of supplement from FBS to HS can have a major impact on cellular functions the effects should be elucidated carefully.

P26 Multicolor Flow Cytometric Analysis of Endothelial Progenitor Cells in the Kidney Excludes their Bone Marrow Origination

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Introduction: Recent literature implies that bone marrow (BM) derived endothelial progenitor cells (EPC) might participate in repair upon renal injury. Most of this data are based on *in vitro* experiments. We have previously shown that mainly endothelial outgrowth cells (EOC, ECFC) can be detected in the kidney after selective endothelial injury. To investigate the origin of such cells *in vivo*, we generated chimeric mice via BM transplantation to trace these cells.

Material and Methods: BM cells were isolated from 8-10 week old ubiquitous tdTomato expressing reporter mice. Recipients were C57Bl/6 mice of the same age. Endothelial injury was induced 6-8 weeks later in 20 of the 25 chimera. 5 mice were used as controls. Kidney, spleen, blood and BM were harvested on days 1, 3, 5 and 7 (n=5 per group) and analyzed using multicolor FACS-analysis and histology. EPC/ECFC ($CD34^+$, $Flk\text{-}1^+$, $CD31^+$, $CD105^+$, $CD146^+$, $CD45^-$, $CD133^-$, $CD115^-$, $CD14^-$), hematopoietic stem cells ($c\text{-}kit^+$ $Sca\text{-}1^+$ lin^-), macrophages ($F4\_80^+$, $CD11b^+$, $CD11c^-$, $GR1^-$), dendritic cells ($CD11c^+$, $CD11b^+$, $GR1^-$), B-cells and T-cells were measured using FACS-analysis.

Results: In injured kidneys the percentage of macrophages (d3: 0.5% vs ctrl: 0.1%), dendritic cells (4.4% vs 1.5%) and T-cells (CD4 0.7% vs 0.2%; CD8 0.3% vs 0.05%) increased significantly on d3 ($p<0.05$). B-cells were unchanged. ECFC were increased on d3 (1.7% vs 0.4% $p<0.001$) and HSC on d1 (0.6% vs 0.2% $p<0.05$). Compared to controls, significantly more tdTomato positive cells were recruited to injured kidneys (d1-d7: 4.3-8.3% vs 2.0%). Almost all macrophages (94.4%±6.4), dendritic cells (96.6%±1.5), neutrophils (99.7%±0.4) and B-cells (99.2%±0.7) were tdTomato positive, while only the minority of T-cells (CD4 34.5%±12.3; CD8 42.0%±11.0) and HSC (14.7%±11.0) were tdTomato positive. A very small proportion of ECFC (0.3%±0.7) were tdTomato positive. Subsequent histological evaluations prove these results.

Conclusions: The present study demonstrates that EPC recruited to the kidney are not of BM origin, while HSC might partially emanate from BM. Further studies will have to define the niche for these progenitor cells in- or outside the kidney.

P27 Possible *in vitro* Differentiation of Adipose Derived Stem Cells into Melanocyte Lineage

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Introduction The broad definition of a stem cell is a cell that has the ability to self-renew and differentiate into one or more specialized terminally differentiated cell types. It has become evident that stem cells persist in, and can be isolated from, many adult tissues. The worldwide epidemic of obesity has caused a surge of interest in the study of adipose tissue biology. Adipose tissue has been shown to contain a population of cells that retain a high proliferation capacity *in vitro* and the ability to undergo extensive differentiation into multiple cell lineages. These cells are referred to as adipose-derived stem cells (ASCS) and are biologically similar, although not identical, to mesenchymal stem cells (MSCs) derived from the bone marrow.

ASCs constitutes a very convenient source of stem cells because they can be easily harvested by liposuction, are available in large numbers, show a strong multi-differentiation ability, attach and proliferate rapidly in culture, making them an ideal cell source in regenerative medicine. Although adipocytes are generally described to derive from mesoderm, recent studies unraveled an unsuspected developmental origin for MSCs and adipocytes in the neural crest.

Vitiligo is a pigmentation disorder caused by the loss of melanocytes. Normal human melanocytes rarely undergo mitosis, and slow recovery of pigmentation with conventional medical therapies has led to the development of surgical treatment methods, including grafting cultured autologous melanocytes. However, it is difficult to obtain sufficient skin tissue for autologous cell transplantation without scar formation. If adipose tissue could be used instead, sufficient amounts of tissue for transplantation would be obtained. Furthermore, ASCs could be isolated and cultured from human adipose tissue.

Aim of the work To evaluate the potential differentiation of adipose derived stem cells into melanocyte precursor cells.

Materials & methods Human subcutaneous adipose tissue was obtained from five female patients (27–40 years old) who underwent section delivery, following their informed consent. All procedures were approved by the ethical committee at Faculty of Medicine, Cairo University. Specimens were collected into a sterile container with HBSS, kept overnight at room temperature.

Separation of the stromal vascular fraction After digestion by collagenase, the ability of lipid-filled adipocytes to float is used to separate them from the stromal vascular fraction.

Culture of stromal stem cells Stromal stem cells, when cultured, adhere to plastic and acquire a fibroblastic-like morphology.

Differentiation of stem cell to melanocyte linage After at least 7 days of culture in Dulbecco's Modified Eagle Medium (DMEM), Human Melanocyte Growth Supplement (HMGS) (Invitrogen) with Medium 254 were used to induce differentiation to melanocyte lineage. HMGS contains all growth factors, hormones, and tissue extracts necessary for the culture of human epidermal melanocytes. Differentiation medium was replaced twice per week for 6 weeks. Differentiation into melanocyte precursor was confirmed by immunocytochemical staining with C-kit & Melanoma Marker (Pan Ab-1 a cocktail of ART-1, MAGE-1, MAGE-3, tyrosinase, gp100, gp75, BAGE-1, and GAGE-1) (Neomarkers, labvision). L-dopa reaction assay was performed for determination of tyrosinase activity.

Results After 2 weeks of culture in the differentiation medium, ASCs began to exhibit melanocytic morphology. Melanocytes were easily identified by their dendritic processes. Quantitative evaluation of melanocytic differentiation was determined 2, 4 and 6 weeks after cell culture in the differentiation medium by counting the number of immuno-positive cells in ten random fields by image analyzer. L- dopa reaction assay revealed evidence of tyrosinase activity that significantly increased by the end of the 6th week.

Conclusion We report the possibility of differentiation of adipose-derived stem cells (ASCs) into Melanocyte precursor cells (MPCs). These preliminary results suggest that ASC- derived-MPCs may be useful for treatment of hypopigmentation-related diseases including vitiligo.

P28 Intra-thecal Transplantation of Autologous Adherent Bone Marrow Cells Induces Histological & Functional Improvement in a Canine Model of Spinal Cord Injury

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Introduction & Aim: Spinal cord injury (SCI) results in demyelination of surviving axons, loss of oligodendrocytes, and impairment of motor and sensory functions. Numerous studies showed that adult bone marrow cells have potential role to enhance tissue repair after injury in different organs. The use of adult bone marrow cells for transplantation in a cell therapy strategy might augment remyelination and improve neurological functions in SCI. To evaluate the possible therapeutic effect of autologous adherent bone marrow cells (ABMCs) on a canine model of induced spinal cord injury.

Material & Methods: Twenty four adult male mixed-breed dogs were divided randomly into three groups; control group, SCI group, & SCI with ABMCs injected group. The last two groups were subjected to a clipping contusion of the spinal cord. All aspects of animal care and treatments were approved by the animal care committee of Cairo University. ABMCs were isolated from the femurs of adult dogs for *in vitro* studies. ABMCs were subjected to flow cytometry, green fluorescent protein (GFP) labeling and neural induction was performed. Intra-thecal transplantation of canine GFP-labeled ABMCs was performed two weeks after SCI. The safety and efficacy of autologous ABMCs therapy were investigated using functional neurological scoring. Furthermore, the differentiation and integration of ABMCs into injured spinal cord were examined using histological and immunohistochemical investigations. Loco-motor performance and functional recovery of hind limbs were evaluated every 4 weeks for 16 weeks after transplantation using a 15-point videotaping scoring system developed for canine SCI. Dogs were scarified after 16 weeks & paraffin embedded spinal cord sections were subjected to routine hematoxylin and eosin (H&E) staining for histological evaluation. For immunohistochemistry, the following primary antibodies were used to evaluate cell differentiation; glial fibrillary acidic protein (GFAP), platelet-derived growth factor receptor-alpha (PDGFR), nestin, neurofilament 70 (NF70) & myelin basic protein (MBP). DAPI was used for nuclear staining. Moreover, electron microscopic study of sections from spinal cords was performed. For quantitative analysis, the number of transplanted GFP labeled ABMCs in the spinal cord was counted. Three sections of spinal cord per antibody were examined for double-positive cells, and four regions per section were evaluated. Myelinated areas and mean area of cavitations from the epicenter of the damaged spinal cord were calculated using image analyzer. For functional evaluation, differences in locomotor scores were analyzed at each time point. All recorded data were subjected to statistical analysis.

Results: Our data demonstrate the presence of a substantial number of GFP-labeled cells in the injured spinal cord up to 16 weeks after transplantation in the sub-acute SCI stage. Less cavitation in the gray and white matter was noticed in ABMCs-treated sections. Numerous GFP positive cells were widely distributed from the epicenter, and were found in the gray and white matter of the injured cord and distributed at the lesion boundary zone, only in ABMCs injected group. GFP+ cells expressing NF70 (a specific marker for mature neurons) were found within cross sections of the ventral roots of ABMCs-treated dogs. Moreover, ABMCs-derived cells positive for both GFP and either the neural progenitor marker nestin, the oligodendrocyte pre-cursor marker PDGFR, or the astrocyte precursor marker GFAP in nerve bundles were demon-strated. Myelination in electron micrographs of sections from autologous ABMCs transplanted dogs demonstrated that remyelinated axons are predominantly from peripheral-like myelin forming cells. Oligodendrocyte-myelinated axons were also observed in smaller axons.

Conclusion: ABMCs therapy in canine SCI model enhanced remyelination, and augmented neural regeneration resulting in improved neurological functions. Therefore, autologous ABMCs therapy might be a safe and promising therapy for spinal cord injuries.

P29 Genome Size Variation in Sexual and Apomictic Boechera Species

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Apomixis is an asexual seed production in which the offspring is a clone of the mother because of the circumvention of meiosis (apomeiosis) and autonomous development of unreduced egg cell (parthenogenesis) without fertilization by sperm nuclei. Apomixis technology is capable of revolutionizing agriculture through fixation of hybrid vigour (heterosis) and other quality traits perpetually in crop generations consequently production of affordable improved seeds. Information on genome size of apomixis model system would facilitate the understanding of mechanism driven its evolution and stability at a molecular level, which holds the key to the integration of apomixis technology into agriculture. Here we have used flow cytometric technique to interrogate 10 sexual and 10 apomictic genotypes belonging to 9 different species for determining genome size variation in diploid Boechera species. Each genotype was represented by 5 plants and the flow cytometric sampling of about 10,000 nuclei/plant was replicated 8 times.

Our data showed a statistically significant difference ($p<0.01$) between the sexual and apomictic genomes. Sexual genotypes recorded an average genome size of 220 Mbp/1C against 232 Mbp/1C for the apomictic species (i.e. difference of about 12 Mbp). Although sexual Boechera had smaller genomes they nonetheless exhibited highest variability in genome size ranging from about 202 Mbp to 248 Mbp compared to apomicts with genome size of between 225 Mbp and 258 Mbp. In addition, we found inter- and intra- specific genome size variation across the two reproductive Boechera species.

Increased genome size in apomictic species compared to sexual counterparts suggest a significant influence of breeding system on mutation accumulation, natural selection pressure and genome size variation in natural plant populations. An excess of about 12 Mbp found in apomictic genotypes signifies extra DNAs (gene copy) that could enhance stability and survival of apomictic Boechera plant species in the absence of syngamy (meiosis).

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….and see you again

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