Gene Function in Cell Growth, Differentiation & Development

Director

Univ.-Prof. Dr. rer. nat.
Martin Zenke

RWTH Aachen University Hospital
Pauwelsstrasse 30, 52074 Aachen

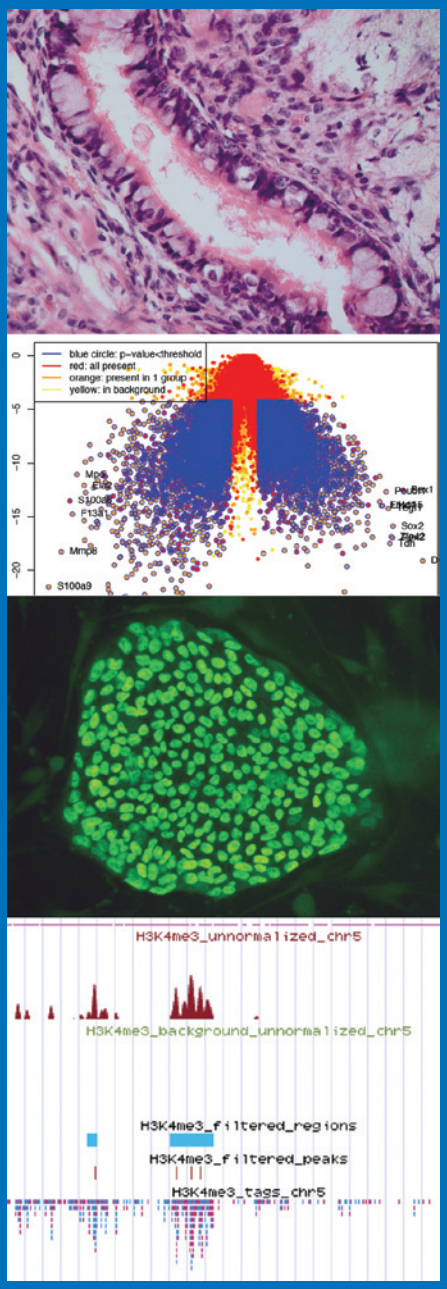
Helmholtz Institute for Biomedical Engineering
Pauwelsstrasse 20, 52074 Aachen

Phone: +49-241-80 80760 (Office)
+49-241-80 80759 (Secretary)
Fax: +49-241-80 82008
Email: martin.zenke@rwth-aachen.de
Web: http://www.molcell.de

Staff

Offergeld, Andrea, Administrative Assistant
Sous, Renate, Administrative Assistant (part time)

Aydin, Gülcan, BSc, Technician
Baek, Jea-Hyun, MSc, PhD Student/Postdoc
Becker, Christiane, Technician
Brosig, Stephanie, Technician
Chauvistré, Heike, MSc, PhD Student
Christ, Anette, MSc, PhD Student
Ding, Xiaolei, MSc, PhD Student/Postdoc
Fleischer, Johannes, MSc Student
Grego, Andrea, BSc Student
Hieronymus, Thomas, PhD, Group Leader
Klisch, Theresa, BSc Student
Lin, Qiong, MSc, PhD Student
Mitzka, Saskia, Technician
Ortseifer, Inga, Dipl.-Ing. (FH), PhD Student
Qin, Jie, MSc, PhD Student
Rehage, Nina, MSc Student
Rogalski, Philipp, MSc Student
Schalla, Carmen, Technician
Schwarz, Sebastian, MSc, PhD Student
Sechi, Antonio, PhD, Group Leader
Seré, Kristin, PhD, Postdoc
Shokouhi, Behnaz, MSc, PhD Student
Sontag, Stephanie, MSc Student
Thönes, Stephan, MSc Student
Wanek, Paul, BSc, Technician
Wang, Xiaoying, MSc, PhD Student
Wolf, Dhana, Student



Stem Cell Biology and Cellular Engineering

Univ.-Prof. Dr. med., Dr. rer. nat.
Wolfgang Wagner

Helmholtz Institute for Biomedical Engineering
Pauwelsstrasse 20, 52074 Aachen

Phone: +49-241-80 88611 (Office)
Fax: +49-241-80 82008
Email: wwagner@ukaachen.de

Hemeda, Hatim, MSc, PhD, Postdoc
Joussen, Sylvia, Technician
Koch, Carmen, MSc, PhD, Postdoc
Lohmann, Michael, MD Student
Moser, Kristin, BSc Student
Reck, Kristina, Student
Schellenberg, Anne, MSc, PhD Student
Walenda, Gudrun, MSc, PhD Student
Walenda, Thomas, MSc, PhD Student/Postdoc
Weidner, Carola, MSc, PhD Student

Introduction

The laboratory has a long standing interest in stem cells, including hematopoietic stem cells (HSC), mesenchymal stem cells (MSC) and embryonic stem cells (ES cells), and their differentiated progeny, such as dendritic cells (DC). Additionally, efforts are directed towards enlarging the developmental potential of somatic cells by employing induced pluripotent stem (iPS) cell technology. HSC and MSC reside in bone marrow in a highly specialized area, referred to as stem cell niche, and in this context we study HSC/MSC interactions in homeostasis, pathology and aging.

Biomedical engineering entails the development of biohybrid systems, comprising cells and engineered materials. Thus, the laboratory investigates the impact of natural and synthetic biomaterials on cell growth, differentiation and function. Furthermore, nanoparticle formulations are developed and used for cell tracking *in vivo* by magnetic resonance imaging (MRI).

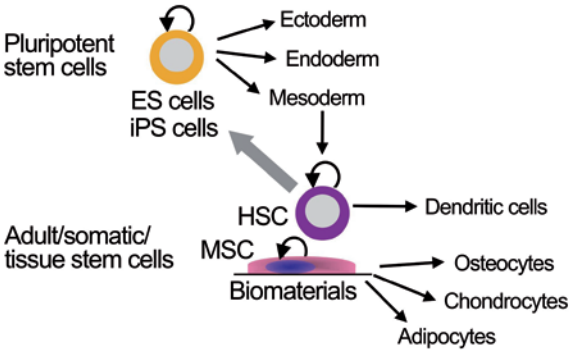


Fig. 1: Pluripotent stem cells include embryonic stem cells (ES cells) and induced pluripotent stem cells (iPS cells). iPS cells are obtained from somatic cells by reprogramming. Hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) reside in the bone marrow niche and develop into all cells of blood and connective tissue, respectively. Biomaterials recapitulate aspects of the niche and influence MSC differentiation.

Dendritic Cell Development and Function are Controlled by Multiple Signalling Pathways

Dendritic cells (DC) represent a population of highly specialized immune cells with pivotal importance for antigen presentation and effector T cell responses. DC develop via multipotent hematopoietic progenitors (MPP) and common DC progenitors (CDP) into conventional DC (cDC) and plasmacytoid DC (pDC) (Fig. 2). We have now analysed the activity of specific signalling pathways on DC subset specification and DC function (Hieronymus et al., 2005; Felker et al., 2010; Seré et al., 2011).

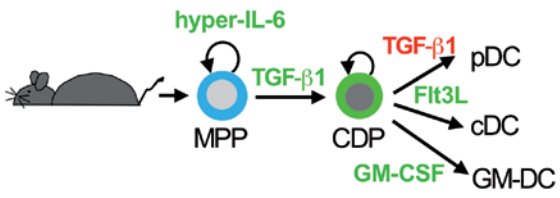


Fig. 2: Influence of hyper-IL-6/gp130, TGF–β1, Flt3 ligand (Flt3L) and GM-CSF signalling on DC lineage commitment and differentiation. Cytokines with promoting and inhibiting activity are depicted in green and red, respectively.

Flt3 ligand (Flt3L) is important for steady state DC development (Fig. 2). The inflammatory cytokine GM-CSF impacts on DC development already at the MPP stage and induced an inflammatory gene signature, including down-regulation of genes important for steady state DC development (Figs. 2 and 3; Seré et al., 2011).

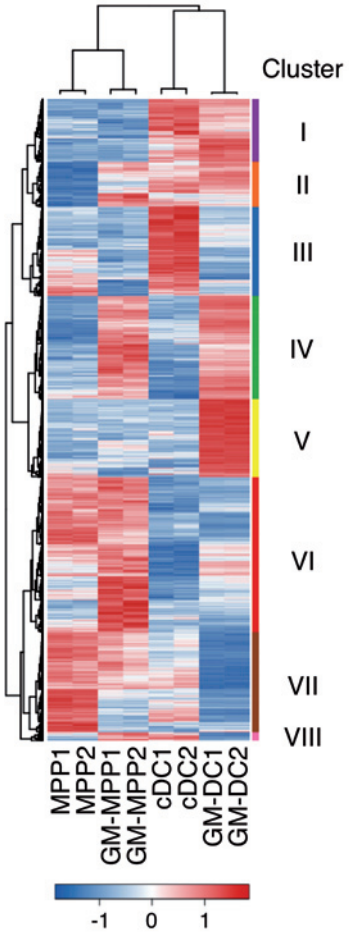


Fig. 3: Hierarchical cluster analysis of gene expression in steady state MPP and cDC, and GM-CSF treated GM-MPP and GM-DC (Seré et al., 2011). Blue and red colours refer to gene expression below and above median, respectively.

Engineering Stem Cells and Their Differentiated Progeny

Pluripotent stem cells, including ES cells and iPS cells, develop into derivatives of all three germ layers (Kim et al., 2008; 2009; and references therein). ES cell differentiation into hematopoietic cells is particularly difficult. We found that forced expression of the polycomb group protein Bmi1 enhances the propensity of ES cells to develop towards cells of the hematopoietic lineage (Fig. 4; Ding et al., 2011). Thus, forced Bmi1 expression provides a mean for derivation of engineered adult stem cells from ES cells.

The recently available methods for reprogramming of adult cells into iPS cells (Kim et al., 2008; 2009; and references therein) offer unique perspectives for disease modelling, drug development and regenerative medicine. The efficient and simultaneous production of large numbers of patient- and disease-specific human iPS cells has remained challenging and a major bottleneck for exploring the potential of iPS cell technology. We address these limitations in the *StemCellFactory* project, which brings together leading experts in stem cell research and engineering sciences, and aims at developing a

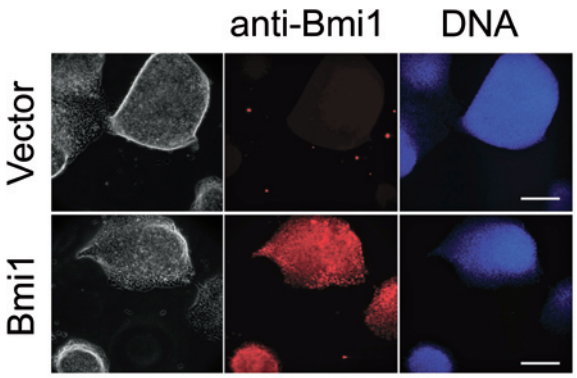


Fig. 4: ES cells were transduced with Bmi1 vector (Bmi1) or empty vector (vector) and stained for Bmi1 with a specific antibody (anti-Bmi1). Cell nuclei were stained with DAPI (DNA) (Ding et al., 2011).

fully automated production process for iPS cells and iPS cell-derived cardiac muscle and neural cells.

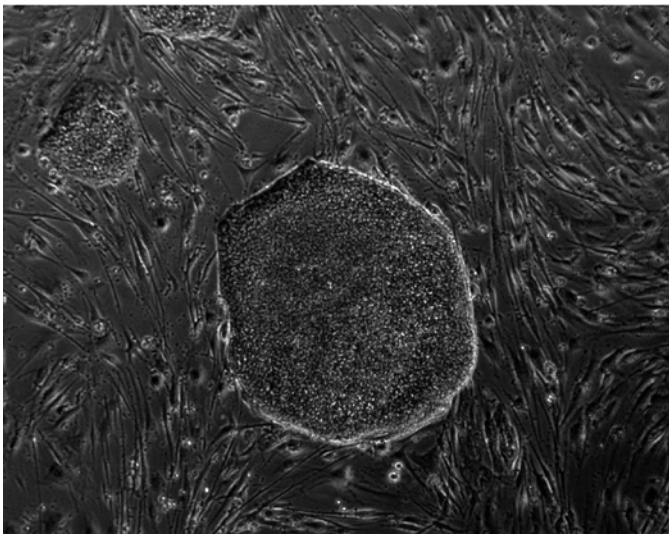


Fig. 5: Human iPS cell colony on mouse embryo fibroblast (MEF) feeder (phase contrast image; in collaboration with O. Brüstle, Life & Brain, Bonn, Germany).

The manufacturing plant integrates automation and standardization of all necessary cell culture steps, as well as a comprehensive quality management. *StemCellFactory* is supported by Bio.NRW and represents the concerted efforts of eight partners in Aachen, Bonn, Leverkusen and Münster (www.stemcellfactory.de).

Iron Oxide Nanoparticles for Cell Tracking by MRI

Tracking of cells following their application *in vivo* is of upmost importance for monitoring efficacy of cellular therapies. Superparamagnetic iron oxid nanoparticles (MNP) possess great potential as contrast agents in MRI due to their transversal (T2 and T2*) relaxation time shortening effects and therefore are frequently used for cell labeling and cell tracking by MRI. We found that modifying MNP shell parameters, such as charge, size, and surface chemistry, using layer-by-layer assembly of polyelectrolytes impact on MNP uptake into cells and intracellular clustering, and thus on MRI contrast properties (Schwarz et al., 2011; in collaboration with W. Richtering, Institute of Physical Chemistry, RWTH Aachen University, Aachen, Germany; U. Himmelreich and M. Hodenius, Biomedical NMR Unit/ MoSAIC, Faculty of Biomedical Sciences and Laboratory of BioNano-Colloids, Interdisciplinary Research Centre, Katholic University Leuven, Belgium; M. Hoehn, In vivo NMR Research Group, MPI for Neurological Research, Cologne, Germany; F. Kiessling, Experimental Molecular Imaging, Helmholtz Institute Aachen, Germany) .

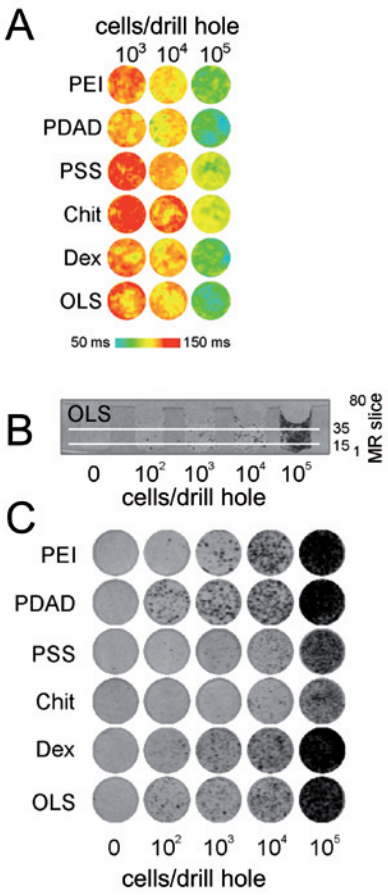


Fig. 6: MRI contrast potential of polyelectrolyte MNP. MNP-labeled DC were filled in drill holes of agarose phantoms and MR images were acquired at 11.7 T. (A) Pseudocolour depiction of T2 relaxation times from quantitative T2 map scans. (B and C) T2-weighted 3D gradient echo MRI. (Schwarz et al., 2011).*

Cellular Aging Determined by Specific DNA Modification

"Cellular aging" of cells in culture has fundamental implications for therapeutic cell preparations. Aging is reflected by changes in cellular morphology, proliferation and differentiation potential. Primary cells can only be expanded for a limited number of passages, until they enter a senescent state and unequivocally stop proliferation. Commonly used parameters for cellular aging are passage number, cumulative population doublings and the time of *in vitro* culture. These parameters need to be carefully documented throughout culture expansion – otherwise it was so far not possible to retrospectively determine the state of cellular aging in cell products.

Recently, we demonstrated that long-term culture of MSC or fibroblasts is associated with specific epigenetic modifications in DNA methylations (Koch et al., 2011a, 2011b; Schellenberg et al. 2011a). Therefore, it was conceivable that methylation at specific cytosine residues provides an epigenetic signature for determining cellular aging. We found that long-term culture can be tracked by a simple method based on continuous DNA methylation changes at six specific CpG sites (Fig. 7). This "Epigenetic

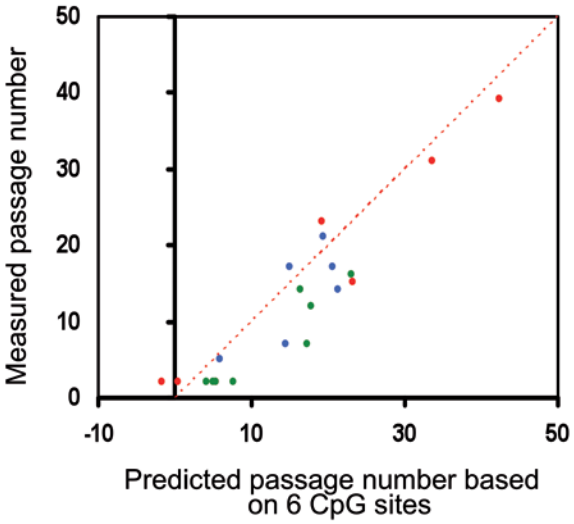


*Fig. 7: **Six CpG sites reflect the number of passages.** Independent cell preparations were used for validation of the "Epigenetic-Signature for Cellular Aging" by pyrosequencing of six specific CpG sites to predict the number of passages (Koch et al., 2011c; red: fibroblasts; blue: AT-MSC; green: BM-MSC.*

Signature for Cellular Aging" can be used as a biomarker for various cell types to predict the state of senescence with respect to the number of passages or days of *in vitro* culture (Patent application: EP 11176593.9).

Biomaterials and Surface Structure Influence Growth of Cells in Culture

MSC have raised high hopes for regenerative medicine and tissue engineering due to their ease of culture expansion, immunomodulatory activity and differentiation potential towards adipogenic, osteogenic and chondrogenic lineages. Frequently, MSC are cultured on plane polystyrene cell culture dishes. In contrast, the microenvironment under *in vivo* conditions is not flat. In cooperation with Fraunhofer Institute for Production Technology (IPT, Aachen) polystyrene micro-structured surfaces with varying groove opening widths and pitches (0.5 – 20μm) were produced to analyze their impact on MSC growth, differentiation potential and replicative senescence.

MSC have been observed to align, elongate and migrate parallel to micro-structured grooves. Moreover, we discovered that proliferation and differentiation capacity of MSC is affected by varying groove size: micro-structured surfaces, which induce a rather round morphology, promoted adipogenic differentiation (Fig. 8), whereas those surfaces, which result in increased cell elongation, enhanced osteogenic differentiation.

Additionally, in further work we identified the synthetic, biodegradable biomaterial Resomer LT706 as being osteoinductive for MSC (Neuss et al., 2011; in collaboration with S. Neuss und W. Jahnen-Dechent, Biointerface Group, Helmholtz Institute for Biomedical Engineering and

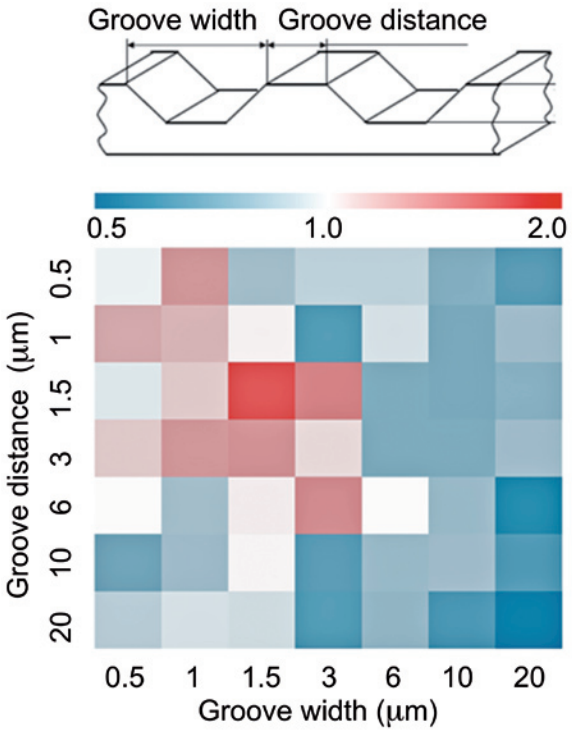


*Fig. 8: Adipogenic differentiation potential of MSC on micro-structured surfaces. **The degree of adipogenic differentiation** was assessed by BODIPY and DAPI staining and normalized to a non-structured polystyrene control and is depicted in heat map format.*

Institute for Pathology, RWTH Aachen; J. Salber, Institute for Technical and Macromolecular Chemistry, RWTH Aachen).

These observations raise potential implications in tissue engineering, since they may provide a non-invasive and biomaterial-based tool to regulate cell function.

Acknowledgements

This work was supported by

- German Research Foundation (DFG)
- German Federal Ministry of Education and Research (BMBF)
- European Union (EU)
- Interdisciplinary Centre for Clinical Research Aachen (IZKF Aaachen)
- Stem Cell Network NRW, Ministry of Innovation, Science, Research and Technology of the State North Rhine-Westphalia
- Excellence Initiative of the German Federal and State Governments
- START-Program of the Faculty of Medicine, RWTH Aachen
- Else Kröner-Fresenius Foundation
- StemCellFactory (BioNRW2), Ministry of Innovation, Science, Research and Technology of the State North Rhine-Westphalia
- Donation by U. L

Selected References in 2011

[1] Bauerschlag, D. O., Ammerpohl, O., Bräutigam, K., Schem, C., Li, Q., Weigel, M. T., Hilpert, F., Arnold, N., Maass, N., Meinhold-Heerlein, I. and Wagner, W. (2011). Progression-free survival in ovarian cancer is reflected in epigenetic DNA methylation profiles. *Oncology* 80,12-20.

[2] Bissels, U., Bosio, A. and Wagner, W. (2011). MicroRNAs are shaping the hematopoietic landscape. *Haematologica.* Nov 4; Epub ahead of print. doi:10.3324/haematol.2011.051730.

[3] Bork, S., Horn, P., Castoldi, M., Hellwig, I., Ho, A. D. and Wagner, W. (2011). Adipogenic differentiation of human mesenchymal stromal cells is down-regulated by microRNA-369-5p and up-regulated by microRNA-371. *J. Cell. Physiol.* 226, 2226-2234.

[4] Cholewa, D., Stiehl, T., Schellenberg, A., Bokermann, G., Joussen, S., Koch, C., Walenda, T., Pallua, N., Marciniak-Czochra, A., Suschek, C. V. and Wagner, W. (2011). Expansion of adipose mesenchymal stromal cells is affected by human platelet lysate and plating density. *Cell Transplant.* 20,1409-1422.

[5] Diehlmann, A., Bork, S., Saffrich, R., Veh, R. W., Wagner, W. and Derst, C. (2011). K_{ATP} channels in mesenchymal stromal stem cells: strong up-regulation of Kir6.2 subunits upon osteogenic differentiation. *Tissue Cell*. 43, 331-336.

[6] Ding, X., Lin, Q., Ensenat-Waser, R., Rose-John, S. and Zenke, M. (2011). Polycomb group protein Bmi1 promotes hematopoietic cell development from ES cells. *Stem Cells Dev.*, May 5. [Epub ahead of print]. DOI: 10.1089/scd.2010.0539.

[7] Ferreira, M. V., Labude, N., Walenda, G., Adamzyk, C., Wagner, W., Piroth, D., Müller, A. M., Knüchel, R., Hieronymus, T., Zenke, M., Jahnen-Dechent, W. and Neuss, S. (2011). *Ex vivo* expansion of cord blood CD34+ cells using IGFBP2 and Angptl-5 impairs sort-term lymphoid repopulation *in vivo*. *J. Tissue Eng. Reg. Med.*, in press.

[8] Horn, P., Bork, S. and Wagner, W. (2011). Standardized isolation of human mesenchymal stromal cells with red blood cell lysis. *Methods Mol. Biol.* 698, 23-35.

[9] Hoss, M., Apel, C., Dhanasingh, A., Suschek, C. V., Hemmrich, K., Salber, J., Zenke, M. and Neuss, S. (2011). Integrin alpha4 impacts on differential adhesion of preadipocytes and stem cells on synthetic polymers. *J. Tissue Eng. Reg. Med.*, in press.

[10] Ko, K., Reinhardt, P., Tapia, N., Schneider, R. K., Araúzo-Bravo, M. J., Han, D. W., Greber, B., Kim, J., Kliesch, S., Zenke, M. and Schöler, H. R. (2011). Evaluating the potential of putative pluripotent cells derived from human testis. *Stem Cells* 29, 1304-1309.

[11] Koch, C. M., Suschek, C. V., Lin, Q., Bork, S., Goergens, M., Joussen, S., Pallua, N., Ho, A. D, Zenke, M. and Wagner, W. (2011a). Specific age-associated DNA methylation changes in human dermal fibroblasts. *PLoS ONE, e16679.*

[12] **Koch, C. and Wagner, W. (2011b). Epigenetic-Aging-Signature to determine age in different tissues.** *Aging* 3: 1-10.

[13] Koch, C., Joussen, S., Schellenberg, A., Lin, Q., Zenke, M. and Wagner, W. (2011c). Monitoring of cellular aging by DNA-methylation at specific CpG sites. *Aging Cell*; in press.

[14] Leisten, I., Kramann, R., Ventura Ferreira, M. S., Ziegler, P., Wagner, W., Neuss, S., Knüchel, R. and Schneider, R. K. (2011). 3D co-culture of hematopoietic stem and progenitor cells and mesenchymal stem cells in collagen scaffolds: An *in vitro* model of the hematopoietic niche. *Biomaterials* 33, 1736-1747.

[15] Moldenhauer, A., Futschik, M., Lu, H., Helmig, M., Götze, P., Bal, G., Zenke, M., Han, W. and Salama, A. (2011). Interleukine 32 promotes hematopoietic progenitor expansion and attenuates bone marrow cytotoxicity. *Eur. J. Immunol.* 41, 1774-1786.

[16] Müller, M., Stockmann, M., Malan, D., Wolheim, A., Tischendorf, M., Linta, L., Katz, S.-F., Lin, Q., Latz, S., Brunner, C., Wobus, A. M., Zenke, M., Wartenberg, M., Böckers, T. M., von Wichert, G., Fleischmann, B., Liebau, S. and Kleger, A. (2011). Ca2+-activated K+-channels - New tools to induce cardiac commitment from pluripotent stem cells in mice and men. *Stem Cell Rev. and Rep.*, Oct 26 [Epub ahead of print] DOI 10.1007/s12015-011-9324-9.

[17] Neuss, S., Denecke, B., Gan, L., Lin, Q., Bovi, M., Apel, C., Wöltfe, M., Dhanasingh. A., Salber, J., Knüchel, R. and Zenke, M. (2011). **Transcriptome analysis of MSC and MSC-derived osteoblasts on Resomer LT706 and PCL: Impact of biomaterial substrate on osteogenic differentiation.** *PLoS ONE,* e23195.

[18] Schellenberg, A., Lin, Q., Schüler, H., Koch, C. M., Joussen, S., Denecke, B., Walenda, G., Pallua, N., Suschek, C. V., Zenke, M. and Wagner, W. (2011a). Replicative senescence of mesenchymal stem cells causes DNA-methylation changes which correlate with repressive histone marks. *Aging* 3, 873-888.

[19] Schellenberg, A., Stiehl, T., Horn, P., Joussen, S., Pallua, N., Ho, A. D. and Wagner, W. (2011b). The composition of subpopulations in mesenchymal stromal cells changes during culture expansion. *Cytotherapy*; Epub ahead of print. doi:10.3109/14653249.2011.640669.

[20] Schellenberg, A., Hemeda, H. and Wagner, W. (2011c). Tracking of replicative senescence in mesenchymal stem cells by colony-forming unit frequency. *Methods Mol. Biol.*; in press.

[21] Schwarz, S., Wong, J. E., Bornemann, J., Hodenius, M., Himmelreich, U., Richtering, W., Hoehn, M., Zenke, M. and Hieronymus, T. (2011). Polyelectrolyte coating of iron oxide nanoparticles for MRI-based cell tracking. *Nanomedicine*, Sep 3. [Epub ahead of print]. Dio:10.1016/j.nano.2011.08.010.

[22] Seré, K. M., Lin, Q., Felker, P., Rehage, N., Klisch, T., Ortseifer, I., Hieronymus, T., Rose-John, S. and Zenke, M. (2011). **Dendritic cell lineage commitment is instructed by distinct cytokine signals.** *Eur. J. Cell Biol.*, Nov 9. [Epub ahead of print]. Doi:10.1016/j.ejcb.2011.09.007.

[23] van de Kamp, J., Kramann, R., Anraths, J., Schöler, H. R., Ko, K., Knüchel, R., Zenke, M., Neuss, S. and Schneider, R. K. (2011). **Epithelial morphogenesis of germline-derived pluripotent stem cells on organotypic skin equivalents** *in vitro*. *Differentiation*, in press.

[24] Walenda, T., Bokermann, G., Ventura-Ferreria, M., Piroth, D., Hieronymus, T., Neuss-Stein, S., Zenke, M., Ho, A. D., Müller. A. M. and Wagner, W. (2011a). Synergistic effects of growth factors and mesenchymal stromal cells for expansion of hematopoietic stem and progenitor cells. *Exp. Hematol.* 39, 617-628.

[25] Walenda, T., Bokermann, G., Jost, E., Galm, O., Schellenberg, A., Koch, C. M., Piroth, D. M., Drescher, W., Brümmendorf, T. H. and Wagner, W. (2011b). Serum after autologous transplantation stimulates self-renewal and proliferation of human hematopoietic progenitor cells. *PLoS ONE* 6:e18012.

[26] Weber, C., Meiler, S., Döring, Y., Koch, M., Drechsler, M., Megens, R. T. A., Rowinska, Z., Bidzhekov, K., Fecher, C., Ribechini, E., van Zandvoort, M. A. M. J., Binder, C. J., Jelinek, I., Hristov, M., Boon, L., Jung, S., Korn, T., Lutz, M. B., Förster, I., Zenke, M., Hieronymus, T., Junt, T. and Zernecke, A. (2011). CCL17-expressing dendritic cells drive atherosclerosis by restraining regulatory T cell homeostasis in mice. *J. Clin. Invest*. 121, 2898-2910.

[27] Wirz, S., Dietrich, M., Flanagan, T. C., Bokerman, G., Wagner, W., Schmitz-Rode, T. and Jockenhoevel, S. (2011). Influence of PDGF-AB on tissue development in autologous platelet-rich plasma gels. *Tissue Eng. Part A* 17,1891-1899.

[28] Würflinger, T., Gamper, I., Aach, T. and Sechi, A. (2011). Automated segmentation and tracking for large-scale analysis of focal adhersion dynamics. *J. Microsc.* 241, 37-53.

[29] Zepeda-Moreno, A., Taubert, I., Hellwig, I., Pietsch, L., Lakshmanan, V. K., Wagner, W. and Ho, A. D. (2011). Innovative method for quantification of cell-cell adhesion in 96 well plates. Cell. Adh. Migr.. 5, 215-219.

Further Reading

Felker, P., Sere, K., Lin, Q., Becker, C., Hristov, M., Hieronymus, T. and Zenke, M. (2010). TGF-beta 1 accelerates dendritic cell differentiation from common dendritic cell progenitors and directs subset specification toward conventional dendritic cells. J. Immunol. 185; 5326-5335.

Hieronymus, T., Gust, T. C., Kirsch, R. D., Jorgas, T., Blendinger, G., Goncharenko, M., Supplitt, K., Rose-John, S., Müller, A. M. and Zenke, M. (2005). Progressive and controlled development of mouse dendritic cells from $Flt3^+CD11b^+$ progenitors in vitro. J. Immunol. 174, 2552-2562.

Kim, J. B., Zaehres, H., Wu, G., Gentile, L., Sebastiano, V., Ko, K., Araúzo-Bravo, M. J., Han, D. W., Ruau, D., Zenke, M. and Schöler, H. R. (2008). Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. Nature 454, 646-650.

Kim, J. B., Sebastiano, V., Wu, G., Araúzo-Bravo, M. J., Sasse, P., Gentile, L., Ko, K., Ruau, D., Ehrich, M., van den Boom, D., Meyer, J., Hübner, K., Bernemann, C., Ortmeier, C., Zenke, M., Fleischmann, B. K., Zaehres, H. and Schöler, H. R. (2009). Oct4-induced pluripotency in adult neural stem cells. Cell 136, 411-419.

Patent applications

Wagner W, Walenda G. Method for cultivating cells in platelet-lysate-containing medium; 2011; EP 11171595.9-2401

Wagner W, Koch CM, Schellenberg A, Joussen S. Senescence-Methylation-Signature; 2011; EP 11176593.9

Team

Above: Stem Cell Biology and Cellular Engineering lab

Right: Anne Schellenberg receives 1st Poster Award at International Stem Cell Conference, Essen, Germany