



Gene Function in Cell Growth, Differentiation & Development

Director

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

RWTH Aachen University Hospital
Pauwelsstrasse 30, 52074 Aachen

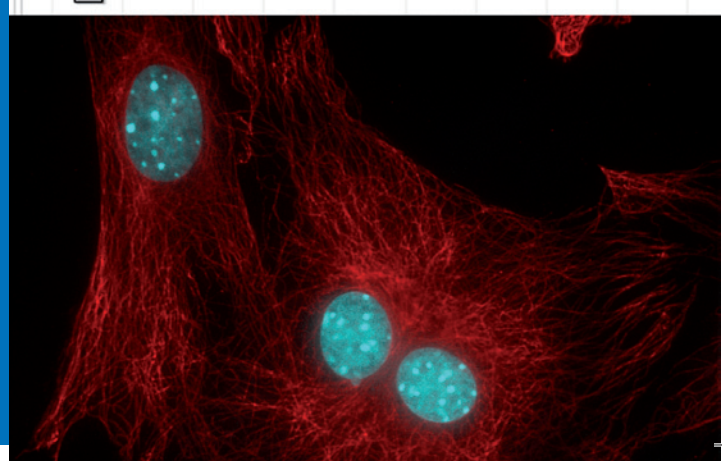
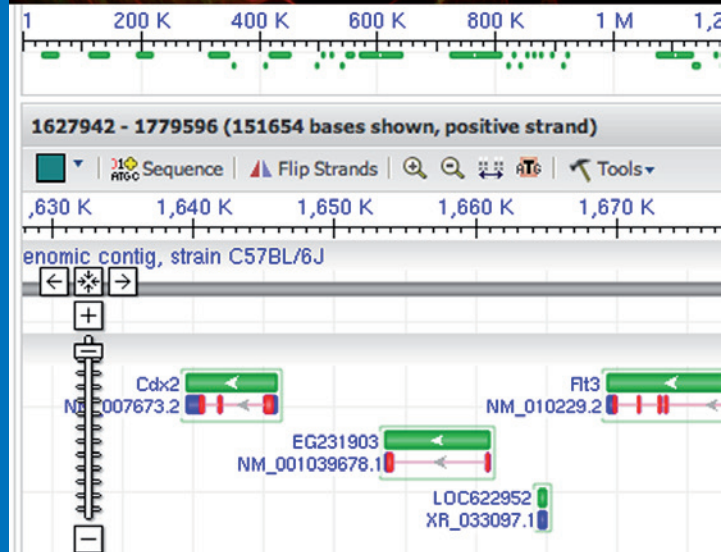
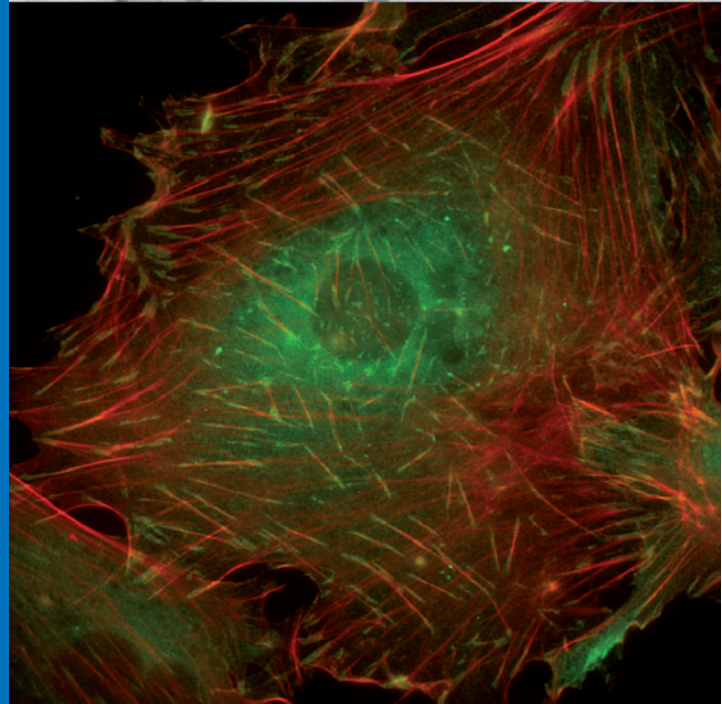
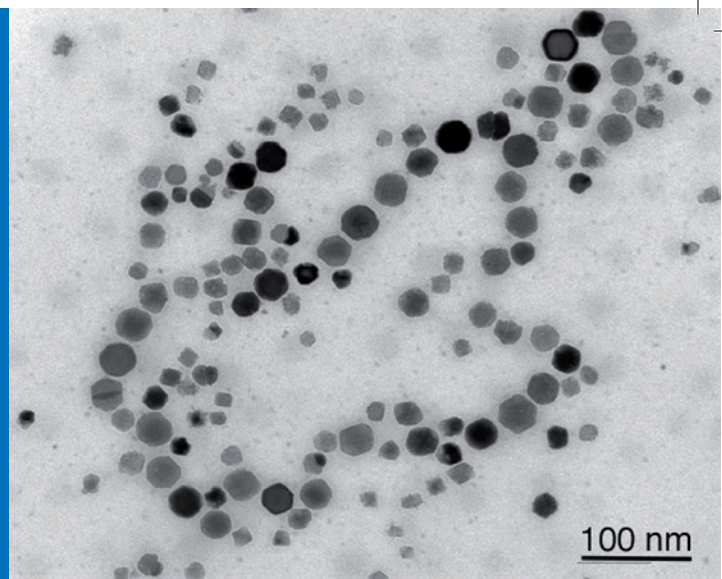
Helmholtz Institute for Biomedical Engineering
Pauwelsstrasse 20, 52074 Aachen

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
Becker, Christiane, Scientific Assistant

Baek, Jea-Hyun, MSc, PhD Student
Bundscherer, Lena, Student
Chauvistré, Heike, MSc, PhD student
Christ, Anette, MSc, PhD student
Ding, Xiaolei, MSc, PhD Student
Döring, Yvonne, MSc, PhD Student
Elbers, Bärbel, Technician
Ensenat-Waser, Roberto, PhD, Postdoc
Gamper, Ivonne, MSc, PhD Student
Guhe, Zita, Student
Hieronymus, Thomas, PhD, Group Leader
Jäntti, Piritta, MSc, PhD Student
Lüneberger, Sigrid, Technician
Ober-Blöbaum, Julia, MSc, PhD Student
Oedekoven, Anne, Technician
Pabich, Julia, Student
Rosewick, Stephanie, Technician
Ruau, David, MSc, PhD Student
Schneider-Kramann, Rebekka, MD, Postdoc
Schröder, Verena, MSc, PhD Student
Schwarz, Sebastian, MSc, PhD Student
Sechi, Antonio, PhD, Group Leader
Seré, Kristin, PhD, Postdoc
Shi, Nian, MSc, PhD Student
Shokouhi, Behnaz, MSc, PhD Student
Siegler, Heike, Student
Simons, Nadine, Technician
Ullrich, Katrin, Student
Wang, Mengxi, Student





Introduction

Cell identity and function are determined by genetic programs, involving a multitude of regulatory circuitries and signalling pathways. Our research focuses on hematopoietic stem/progenitor cells and their differentiated progeny, and how cell fate and function are specified. Stem cells represent a particular attractive cell type for studying cell fate decisions, since they combine two unique properties in one cell: a high self-renewal activity and a broad differentiation potential, which puts stem cells aside from most other somatic cells.

Stem/progenitor cells are isolated from human cord blood, bone marrow or peripheral blood and from mouse bone marrow, and cells are grown with specific cytokines. Cells are then induced to differentiate with yet another set of cytokines and/or differentiation factors, and the ongoing changes in gene expression are monitored on a genome-wide scale by DNA microarrays. The development of antigen presenting dendritic cells (DC) and red blood cells is examined in detail. Mouse embryonic stem cells (ES cells) are also studied. Genes with a determining role in cell fate decisions and cell functions are analyzed in vitro and in vivo in knockout approaches and knockout mouse models. Cells are transplanted into recipient mice and synthetic nano-sized particles are employed for monitoring cell position and function by magnetic resonance imaging (MRI) in vivo.

Biomedical engineering involves the development of biohybrid systems containing cells and bioengineered scaffolds. In this context we study the impact of natural and synthetic biomaterials on cell differentiation and function, including the induction of unwanted immune responses elicited by DC activation. Cells are endowed with novel and wanted properties by standard technology of genetic engineering and implanted in biohybrid systems for use in medical therapy.

In 2008 the institute received generous support by a private donation to foster stem cell research of junior investigators.

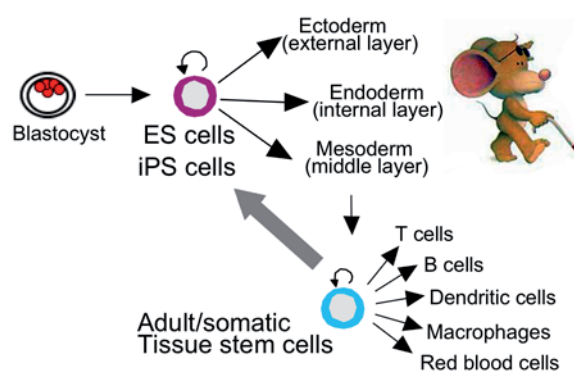


Figure 1: Embryonic stem (ES) cells are derived from the inner cell mass of blastocyst and give rise to cells of all three germ layers (ectoderm, endoderm and mesoderm). Hematopoietic stem cells represent adult/somatic stem cells and develop into all mature blood cells. Adult/somatic stem cells (tissue stem cells) can be reprogrammed to acquire ES cell-like properties (arrow), referred to as induced pluripotent stem (iPS) cells.

Molecular Determinants of Red Blood Cell Development

Red blood cells represent one of the most abundant specialized cell types in the human body. They develop from hematopoietic stem/progenitor cells in bone marrow in response to various stimuli, including thyroid hormone (3,5,3'triiodo-L-thyronine, T₃; Bartunek and Zenke, 1998; Figure 2). T₃ exerts its activities by binding to thyroid hormone receptors (TR), which are ligand-dependent transcription factors that activate T₃ responsive target genes.

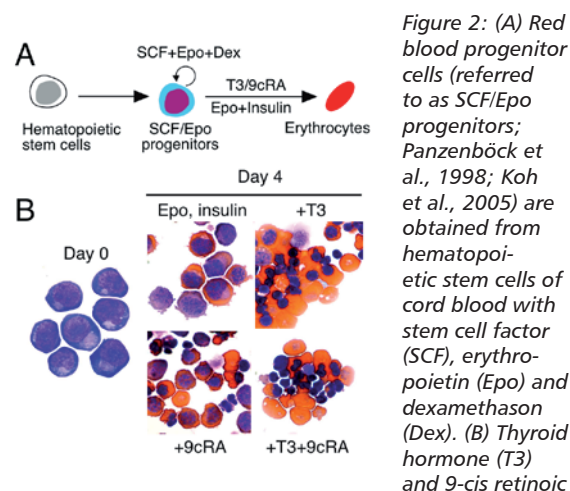


Figure 2: (A) Red blood progenitor cells (referred to as SCF/Epo progenitors; Panzenböck et al., 1998; Koh et al., 2005) are obtained from hematopoietic stem cells of cord blood with stem cell factor (SCF), erythropoietin (Epo) and dexamethason (Dex). (B) Thyroid hormone (T₃) and 9-cis retinoic acid (9cRA) accelerate differentiation of SCF/Epo cells into fully mature red blood cells (orange staining; Gamper et al., 2009).

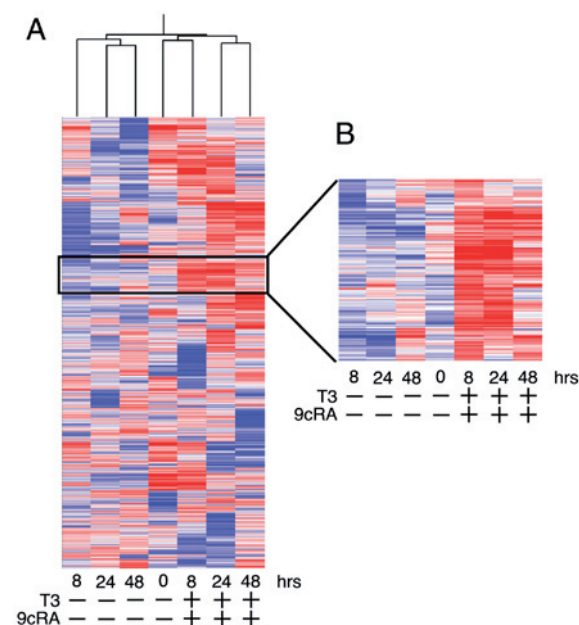


Figure 3: SCF/Epo cells were differentiated with and without T₃+9cRA and subjected to genome-wide gene expression profiling and hierarchical cluster analysis (A and B). Each row represents one gene and color indicates expression variation: red, higher expression and blue, lower expression in relation to the mean expression value of the respective gene (Gamper et al., 2009).



By employing in vitro culture systems (Panzenböck et al., 1998; Koh et al., 2005) and genome-wide gene expression profiling with DNA microarrays we searched for T3 responsive genes (Gamper et al., 2009; Figures 3 and 4). Bioinformatics identified GAR22 (growth arrest specific 2 (GAS2)-related gene on chromosome 22) as a novel and direct target gene of ligand-activated TR. GAR22 was originally found as a putative tumor suppressor gene but its mode of action is unknown. Ectopic expression of GAR22 in red cell progenitors caused growth inhibition and withdrawal from cell cycle and this is suggested to induce and/or contribute to red blood cell development.

Surprisingly, somatic cells can be reprogrammed to acquire pluripotency and ES cell-like properties by expression of a defined set of transcription factors, including Oct4, Sox, c-Myc and Klf4 (referred to as induced pluripotent stem cells, iPS cells; Figure 1). We have found that mouse neural stem cells endogenously express the two reprogramming factors Sox2 and c-Myc (Ruau et al., 2008; Kim et al., 2008). Thus, iPS cells were derived from adult neural stem cells by the expression of Oct4 together with either Klf4 or c-Myc (Kim et al., 2008). These two-factor (2F) iPS cells contributed to development of the germline and formed chimeras.

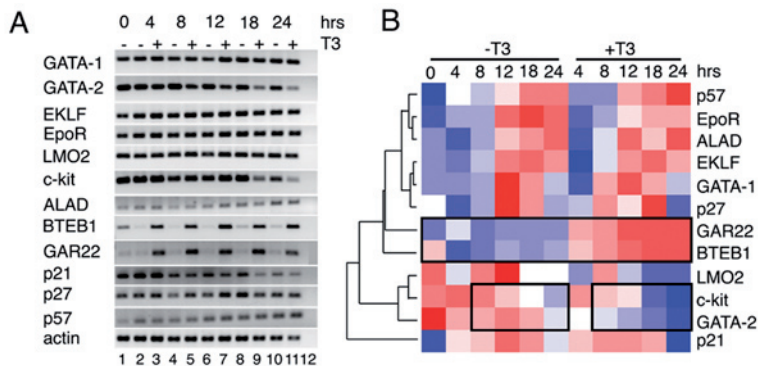


Figure 4: The impact of T3 on red cell gene expression. (A) Semiquantitative RT-PCR of red cell genes and analysis in agarose gels. (B) Quantitative RT-PCR and hierarchical cluster analysis of T3 induced genes (GAR22 and BTEB, box) in heat map format (color code as in Figure 3; Gamper et al., 2009).

Genome-wide gene expression profiling demonstrates that 2F iPS cells are similar to ES cells at the molecular level (Figures 5 and 6; Kim et al., 2008; in collaboration with H. R. Schöler, MPI for Molecular Biomedicine, Münster, Germany). Thus, endogenous expression of Sox2 and c-Myc complements the 2F reprogramming process. Subsequent work demonstrated that expression of Oct4 only yields 1F iPS cells, indicating that Oct4 is both required and sufficient to generate iPS cells from adult neural stem cells (Kim et al., 2009).

Genomics of Pluripotency

Specific stem cell types with distinct developmental potential occur during development: (i) transient pluripotent stem cells in blastocysts that differentiate into derivatives of all three germayers (ectoderm, endoderm and mesoderm) and (ii) tissue resident stem cells (adult/somatic stem cells) with a more restricted potential that give rise to all cells of a given tissue or organ (Figure 1).

Nanoparticles for Cell Tracking in vivo

Accurate delivery of cells to target organs is crucial for successful cell-based therapy with stem cells and immune cells, including antigen presenting dendritic cells (DC; Zenke and Hieronymus, 2006). Labeling of cells with nano-sized contrast agents provides a powerful means of monitoring cell position and function by magnetic resonance imaging (MRI). Thus, we have used a panel of formulations to generate nanoparticles for cell tracking in vivo by MRI (Himmelreich et al., 2006; Schwarz et al., 2009; in collaboration with M. Hoehn, MPI for Neurological Research, Cologne, Germany; U.

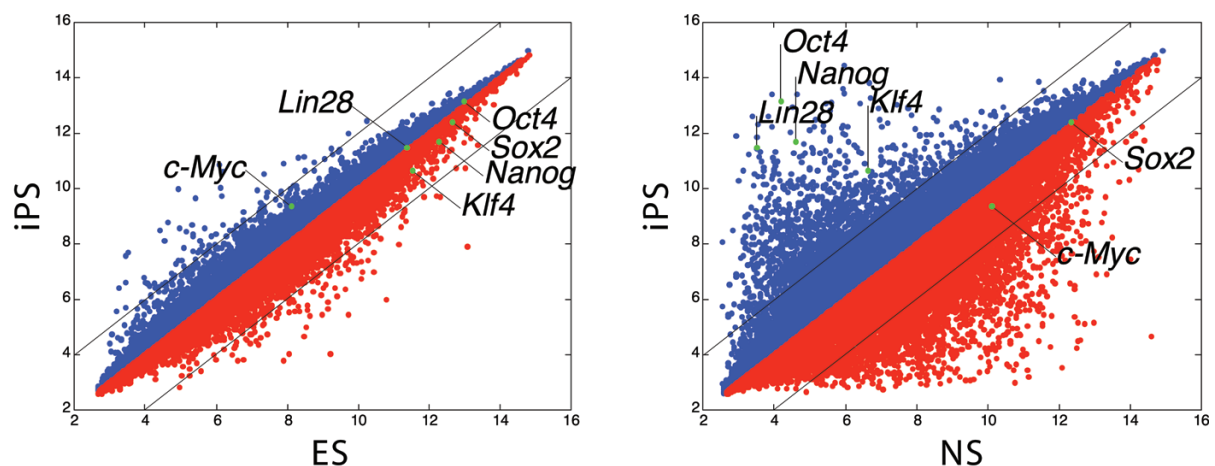


Figure 5: 2F iPS cells (iPS), ES cells (ES) and neural stem cells (NS) were subjected to genome-wide gene expression profiling and scatter plot analysis. Positions of the pluripotency

genes Oct4, Nanog, Sox2, c-Myc, Klf4 and Lin28 are indicated. Note the highly related gene expression pattern of 2F iPS cells and ES cells (left panel) (Kim et al., 2008).

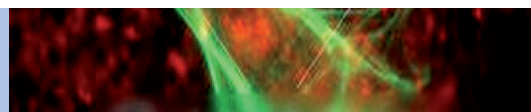
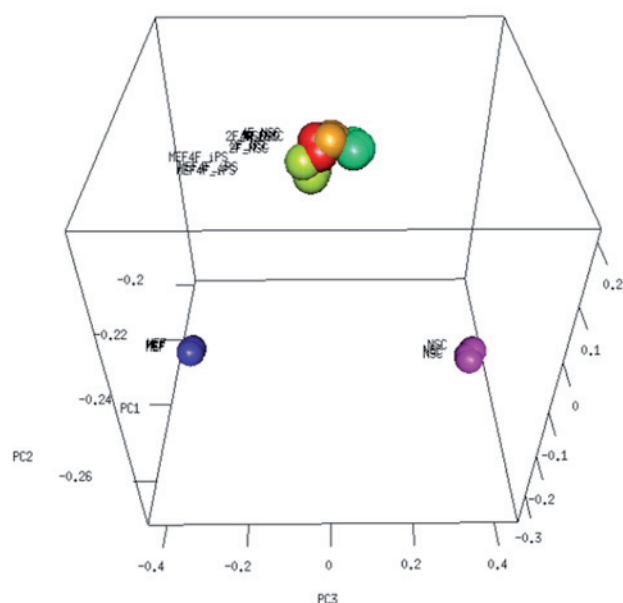


Figure 6: Principal component analysis (PCA) of somatic cells (mouse embryo fibroblasts, MEF and neural stem cells, NSC; blue and purple, respectively) and pluripotent cells (ES cells, 4F iPS cells from MEF, 2F iPS cells and 4F iPS cells from NSC; turquoise, green, red and orange, respectively). Each individual data point corresponds to the genome-wide analysis of the respective cell type (37,000 data points). Duplicates and triplicates are shown.



Bergemann, Chemicell, Berlin, Germany; E. O. Reimhult, ETH, Zürich, Switzerland; W. Richtering, Institute for Physical Chemistry, M. Hodenius and T. Schmitz-Rode, Applied Medical Engineering, Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany).

We investigated the uptake of synthesized or bacterial magnetic nanoparticles (MNP) into hematopoietic Flt3⁺ stem cells and DC from mouse bone marrow (Hacker et al., 2003; Hieronymus et al., 2005). We show that (i) uptake of both synthetic and biogenic nanoparticles into cells endow magnetic activity and (ii) low numbers of MNP loaded cells are readily detected by MRI in agarose phantoms in vitro (Figure 7) and following adoptive transfer in mice in vivo (Figure 8).

Himmelreich, Catholic University of Leuven, Belgium; S. Aime, Department of Chemistry, University of Torino, Italy; D. Schueler, Department of Microbiology, Ludwig-Maximilians-University, Munich, Germany; C.

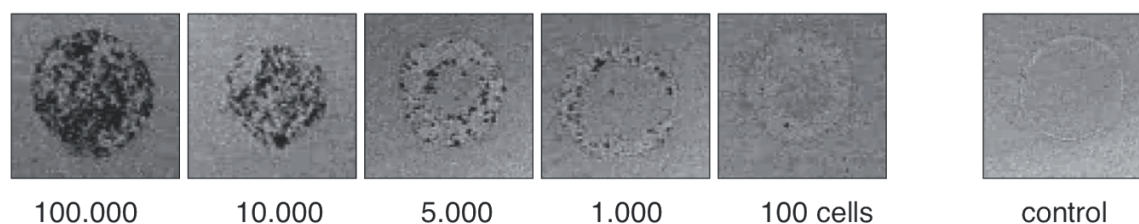


Figure 7: Sections of T2* 3D gradient echo MR images of MNP-labeled DC in agarose phantoms (Schwarz et al., 2009).

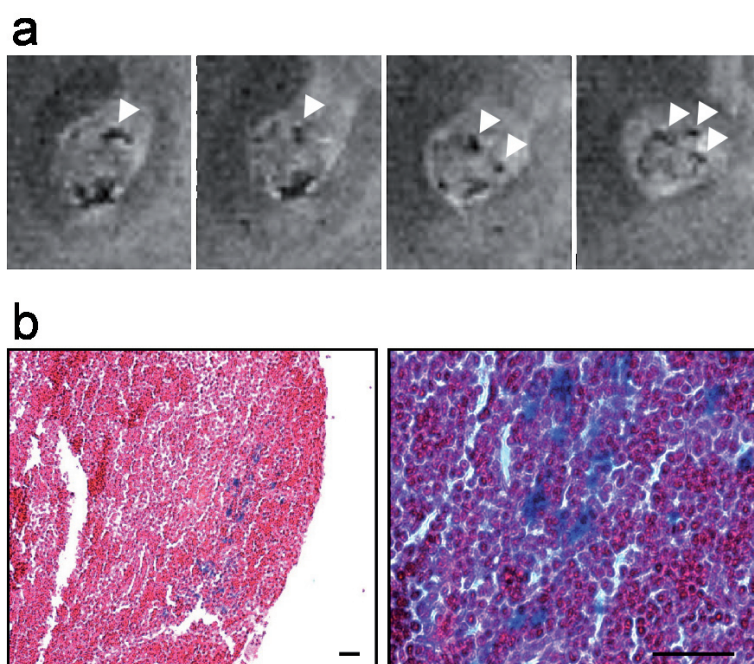


Figure 8: Detection of MNP-labeled DC after migration to draining lymphnodes. (a) T2*-weighted 3D gradient echo MR images of a single inguinal draining lymphnode. Arrowheads indicate intranodal signal reduction. (b) Histochemical staining with Prussian-blue of lymphnode as in (a). Right panel, 4 x higher magnification of detail in left panel (Schwarz et al., 2009).



Figure 9: PhD student Jea-Hyun Baek receives Good Paper Award at International Dendritic Cell Conference DC 2008, Kobe, Japan.

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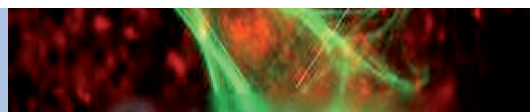


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Further reading

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Team

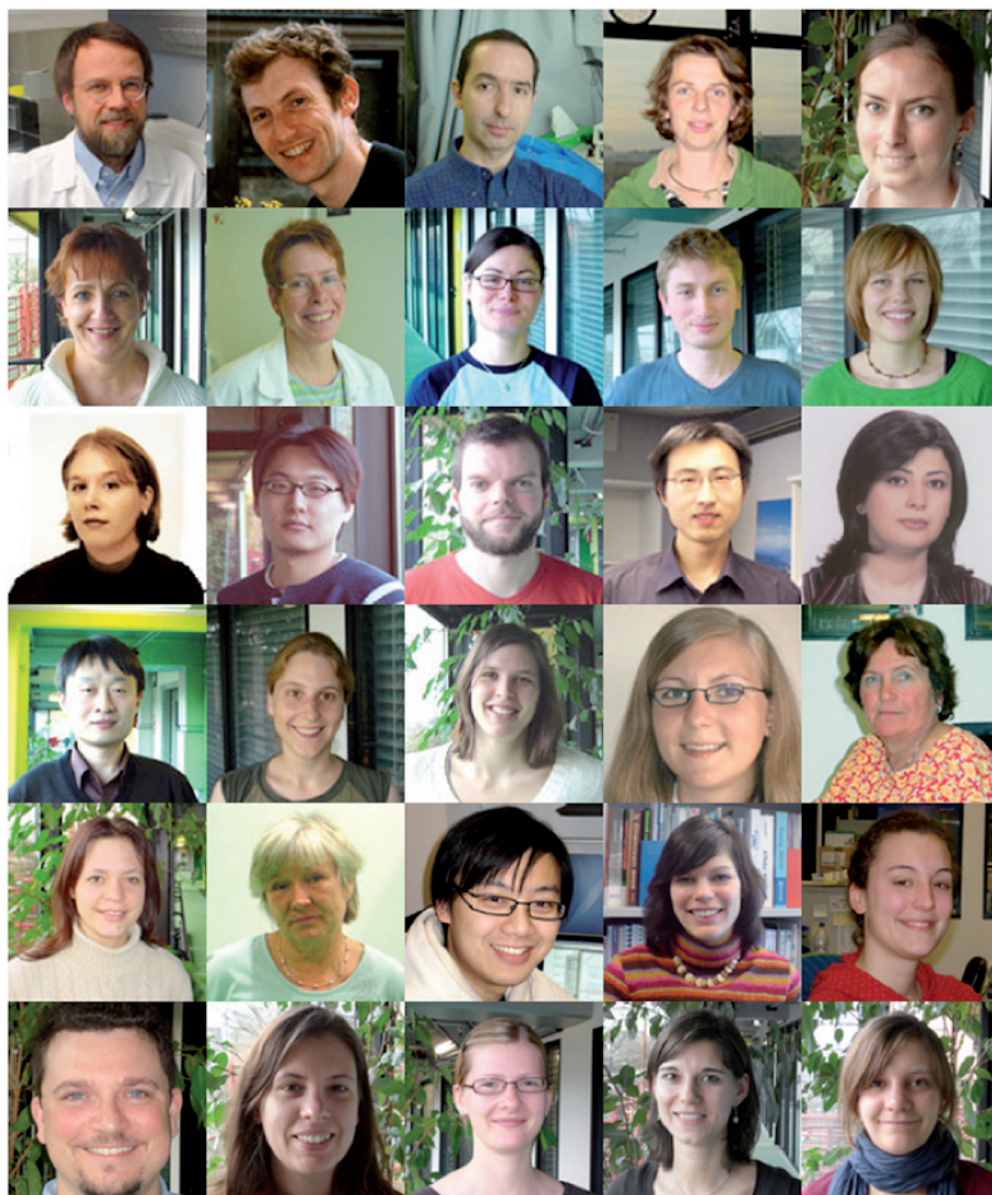


Figure 10: Labout 2008 of Cell Biology and Biointerface groups at Heidelberg Castle.