Gene Function in Cell Growth, Differentiation & Development

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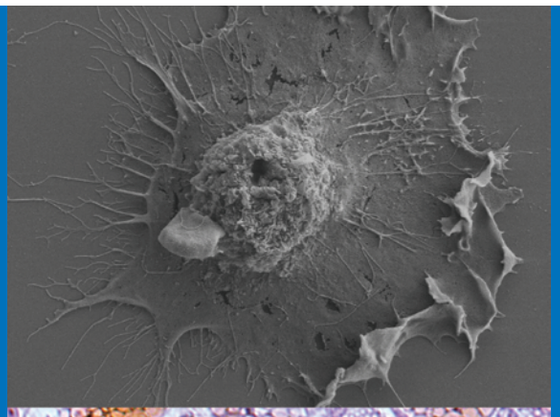

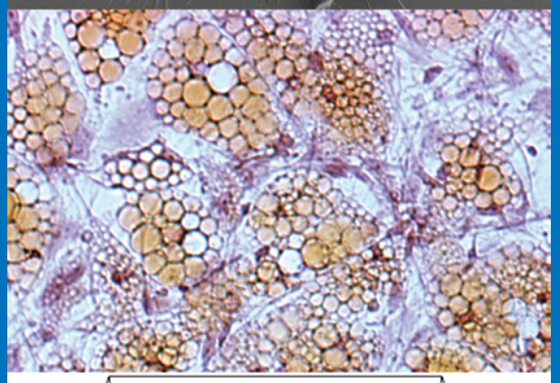

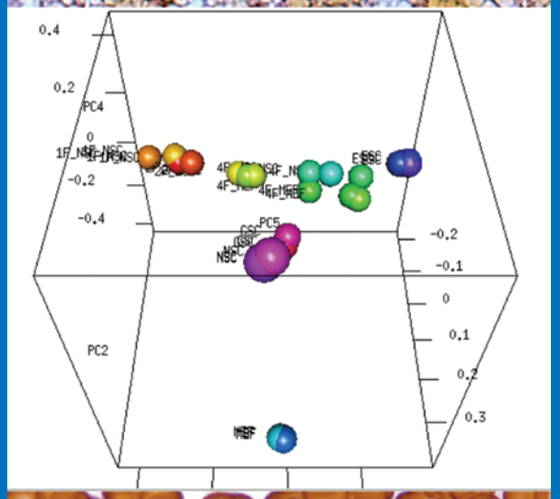

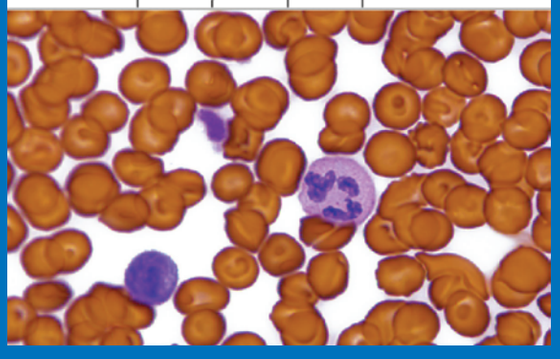

Stem Cell Biology and Cellular Engineering

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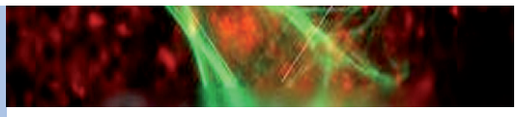


Introduction

Genetic programs determine cell identity and function and thus cells are now being engineered to acquire novel and wanted identities and functions. The laboratory studies the developmental potential of stem cells, including hematopoietic stem cells (HSC), mesenchymal stem cells (MSC) and embryonic stem cells (ES cells), and their differentiated progeny. In addition, efforts are directed towards enlarging the potential of somatic cells by employing various reprogramming strategies, such as 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 involves the development of biohybrid systems comprising cells and engineered synthetic materials. Thus, the laboratory investigates the impact of natural and synthetic materials and of engineered nanoparticles on cell differentiation and function. This also includes studies on unwanted immune responses elicted by antigen presenting dendritic cells (DC). Nanoparticles are used for monitoring cell migration and function by magnetic resonance imaging (MRI).

In 2009 the institute welcomed Wolfgang Wagner, MD, PhD from the Ruprechts-Karls-University Heidelberg, Germany who took up his position as a Professor for Stem Cell Biology and Cellular Engineering. The group of Wolfgang Wagner receives core funding from the Stem Cell Network North Rhine Westphalia (NRW), Ministry of Innovation, Science, Research and Technology NRW, Düsseldorf, Germany.

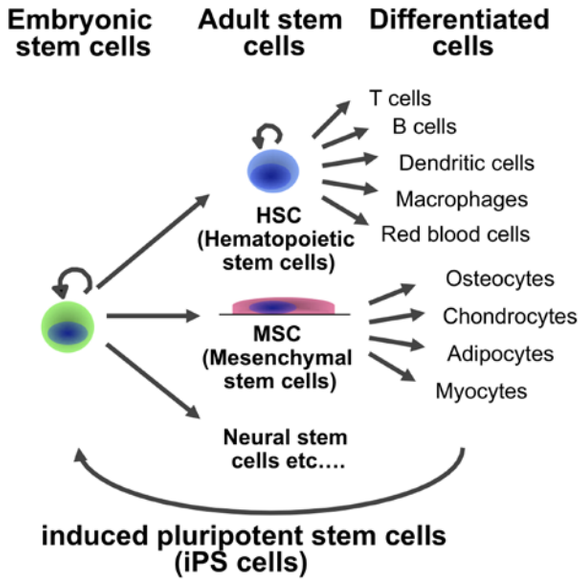


Fig. 1: Embryonic stem cells (ES cells) are pluripotent and give rise to cells of all three germ layers, including hematopoietic cells and mesenchymal cells. Hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) develop into all cells in blood and connective tissue, respectively. Differentiated cells can be reprogrammed to acquire pluripotency and properties of ES cells, referred to as induced pluripotent stem cells (iPS cells).

Antigen Presenting Dendritic Cells

Antigen presenting dendritic cells (DC) represent highly specialized immune cells with a central role in immunity and tolerance induction. DC sense antigens, which are taken-up, processed and presented in the context of MHC molecules to elicit antigen specific T cell responses (Zenke and Hieronymus, 2006). Specific DC subsets exists that differ in surface phenotype, function, activation state and anatomical localization. The main DC subsets are (i) tissue/interstitial DC in organs, now referred to as conventional DC (cDC); (ii) plasmacytoid DC (pDC) in blood that represent the major producers of type I interferon (iii) CD8α DC in lymphoid tissue and (iv) Langerhans cells (LC), the cutaneous contingent of DC in epidermis.

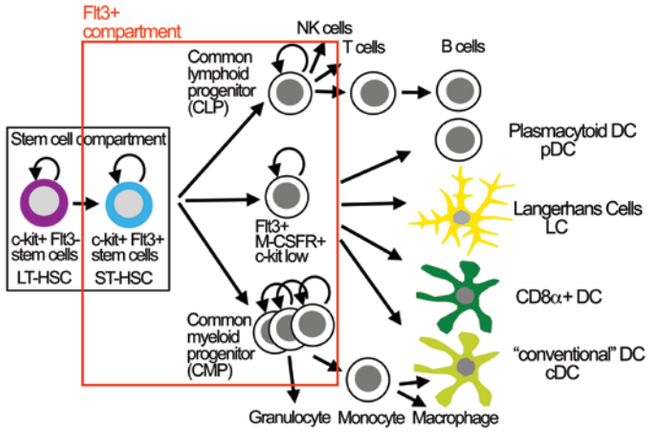


Fig. 2: Hematopoietic stem cells (HSC) give rise to all mature cells in blood and to blood-borne cell in peripheral lymphoid organs. DC subsets develop from HSC through consecutive steps of lineage commitment and differentiation.

All DC subsets develop from hematopoietic stem cells via Flt3 expressing progenitors through consecutive steps of lineage commitment and differentiation (Fig. 2). Surprisingly, DC development shows remarkable plasticity and DC can develop from both lymphoid and myeloid compartments. Additionally, a clonogenic DC progenitor for cDC and pDC was identified, referred to as Flt3+M-CSFR+c-kitlow common DC progenitor (CDP). The laboratory studies DC development by employing *in vitro* culture systems and knockout mouse models (Hacker et al., 2003; Hieronymus et al., 2005; Ju et al., 2007).

Cell-based therapies, including immunotherapy with DC, require accurate delivery of cells to target tissue and monitoring cell position over extended periods of time. Therefore, DC were labelled with nano-sized magnetic iron oxide nanoparticles (MNP) for monitoring DC migration and position by MRI (Schwarz et al., 2009; in collaboration with M. Hoehn, MPI for Neurological Research, Cologne, Germany; U. Himmelreich, Catholic University of Leuven, Belgium; S. Aime, University of Torino, Italy; D. Schueler, Department of Microbiology, Ludwig-Maximilians-University, Munich, Germany; C. Bergemann, Chemicell, Berlin, Germany; 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). First, we investigated the uptake of synthetic MNP or bacterial magnetosome MNP into DC. Second, we determined detection limits of MNP-loaded DC in agarose phantoms (Fig. 3). It was found that DC readily engulf MNP and MNP-labeled DC were detected by MRI. Detection limit was 100 MNP-labeled DC (Fig. 3). Prussian Blue staining confirmed the presence of iron oxide in DC (Fig. 4).

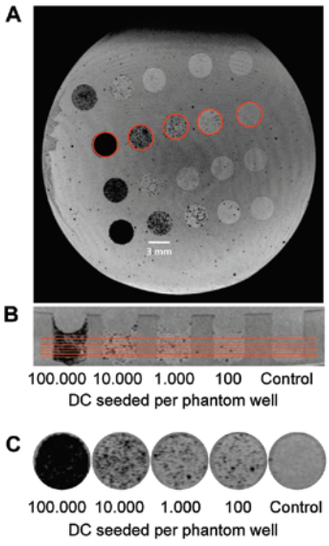


Fig.3: Agarose phantoms of MNP-loaded DC detected by 11.7 T MRI (Schwarz et al., 2009).

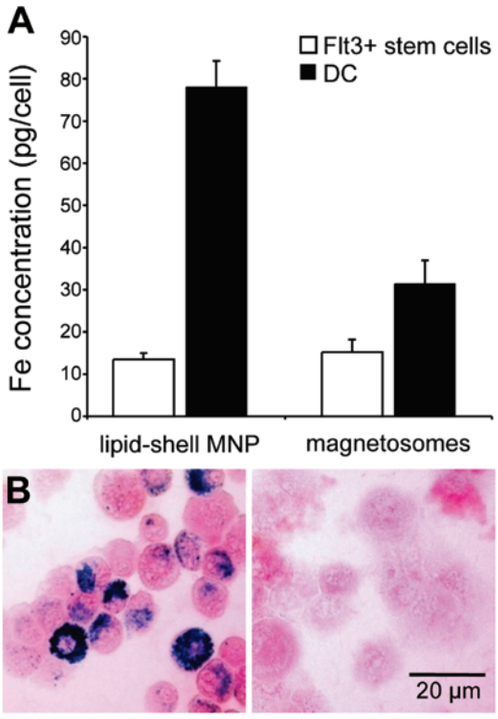


Fig. 4: Lipid-shell MNP and bacterial magnetosome MNP are taken-up by DC. Iron concentration per cell and iron localization in DC by histological staining are shown (A and B, respectively).

Reprogramming and Induction of Pluripotency

Recent progress in stem cell biology demonstrates that somatic cells can be readily reprogrammed and induced to acquire pluripotency by a defined set of transcription factors, including Oct4, Sox2, c-Myc and Klf4 (referred to as induced pluripotent stem cells, iPS cells; Fig. 1).

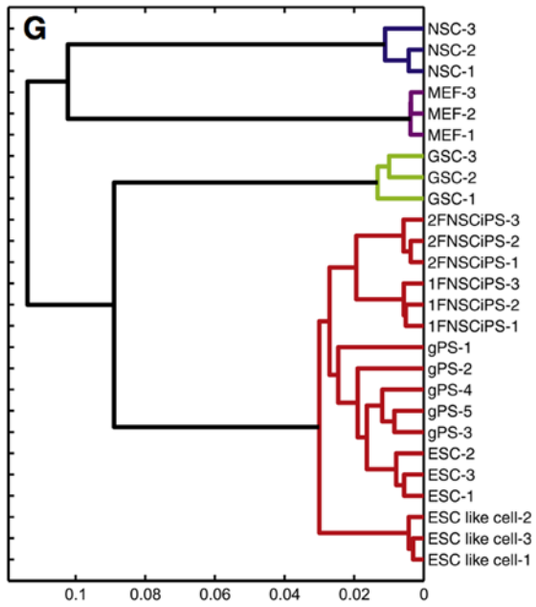


Fig. 5: Hierarchical cluster analysis of genome-wide gene expression by DNA microarrays. MEF, mouse embryo fibroblasts; 1 factor and 2 factor NSC-derived iPS cells, 1FNSCiPS and 2FNSCiPS, respectively; ES cells, ESC (Ko et al., 2009). Pluripotent cells (ESC, iPS cells and gPS cells) cluster together. GSC form a cluster, which is distinct from somatic cells (MEF and NSC).

We have found that mouse neural stem cells (NSC) 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 NSC by the expression of Oct4 together with either Klf4 or c-Myc (Kim et al., 2008). Additionally, subsequent work demonstrated that Oct4 alone is sufficient for reprogramming of NSC (Kim

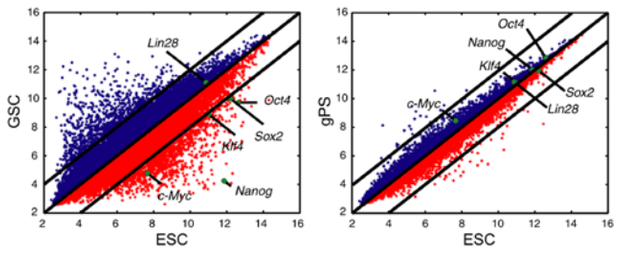
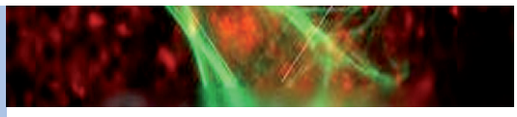


Fig.6: Global gene expression of GSC, ESC and gPS cells is depicted by scatter plot analysis (Ko et al., 2009). A panel of pluripotency-associated genes are indicated. Please note the high similarity of gPS cells and ES cells (ESC).



et al., 2009). Germline-derived stem cells (GSC) express Oct4 and thus GSC can be induced to acquire pluripotency without exogenous transcription factors by employing specific culture conditions (referred to as germline-derived pluripotent cells, gPS cells; Ko et al., 2009). gPS cells exhibit a gene expression repertoire which is very similar to ES cells (Figs. 5 and 6; in collaboration with H. R. Schöler, MPI for Molecular Biomedicine, Münster, Germany). Pluripotency of gPS cells was confirmed by *in vitro* and *in vivo* differentiation, including germ cell contribution and transmission (Ko et al., 2009).

Molecular Analysis of Adult Stem Cells

Throughout life regeneration of tissues is facilitated by stem cells, referred to as adult/somatic/tissue specific stem cells. Stem cells have the dual capacity (i) of differentiating into specific cell types and (ii) of self-renewing to maintain the stem cell pool. These functions have to be tightly regulated according to the physiologic needs of the organism. There is a growing perception that direct cell-cell contacts, referred to as "stem cell niche", plays a central role for stem cell maintenance and regulation. Our bone marrow for example harbours two types of adult stem cells: (i) HSC that give rise to all cell types in blood and blood-borne lymphoid organs and (ii) MSC that resemble progenitors for bone, fat and cartilage (Figs. 1 and 7). Our group investigates molecular properties of HSC and MSC and mechanisms that govern the balance between self-renewal and differentiation. Additionally, we study the HSC/MSC interplay and the adhesion molecules involved (Walenda et al., 2009; Wein et al., 2010; Fig. 7).

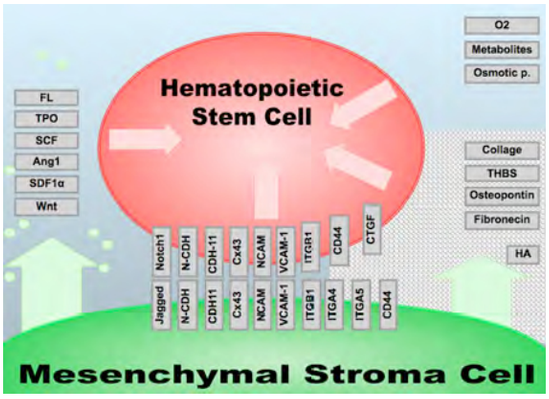


Fig. 7: The stem cell niche in bone marrow and molecules involved in HSC/MSC interactions.

MSC are currently being tested in more than hundred clinical trials. Hope in regenerative medicine has been fueled by novel insights from stem cell biology, new molecular tools and promising preclinical models. At the same time, there is a growing perception that standardized culture conditions and reliable methods for quality control of MSC are urgently needed.

Our group gained insight into how culture media, biomaterials and cell culture techniques affect the composition of therapeutic cell preparations (Wagner et al., 2009; 2010). For example, culture-expansion of MSC over long periods of time impacts on cell proliferation and differentiation potential. Microarray analysis revealed that this is accompanied by continuous and organized gene expression changes. Following cell passaging genes involved in cell division and DNA repair are down regulated whereas others are higher expressed (Bork et al., 2010; Teschendorff et al., 2010; Fig. 8). These long-term culture associated gene expression changes are also observed in independent donor samples and even by cross-validation between different laboratories (Schallmoser et al., 2010). Therefore, it is conceivable to use a specific panel of gene expression markers for quality control of MSC.

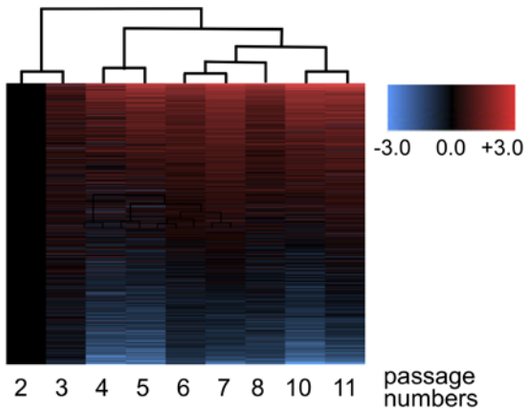


Fig. 8: Human MSC were expanded for 11 passages (up to three months) and subjected to gene expression profiling with DNA microarrays. Hierarchical cluster analysis of gene expression profiles revealed continuous changes with higher passages (red and blue, higher and lower gene expression than median, respectively).

Acknowledgements

This work was supported by

- German Research Foundation (DFG)
- German Federal Ministry of Education and Research (BMBF)
- Interdisciplinary Centre for Clinical Research on Biomaterials and Implants (IZKF BioMAT)
- Stem Cell Network NRW, Ministry of Innovation, Science, Research and Technology of the State Nordrhein-Westfalen
- Erasmus Program of the European Community
- Donation by U. L.

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Team

Fig 9: Stem Cell Biology and Cellular Engineering lab.