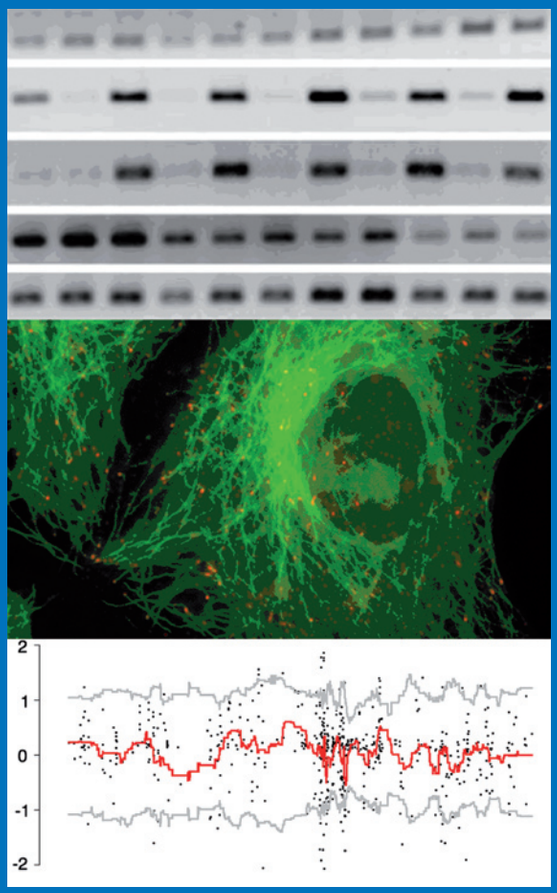
Gene Function in Cell Growth, Differentiation & Development



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Introduction

Intricate circuitries of genes determine cell development, identity and function. The activities of the laboratory focus on 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, such as antigen presenting dendritic cells (DC). In addition, efforts are directed towards enlarging the 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. This also includes studies on unwanted immune responses elicted by antigen presenting dendritic cells.

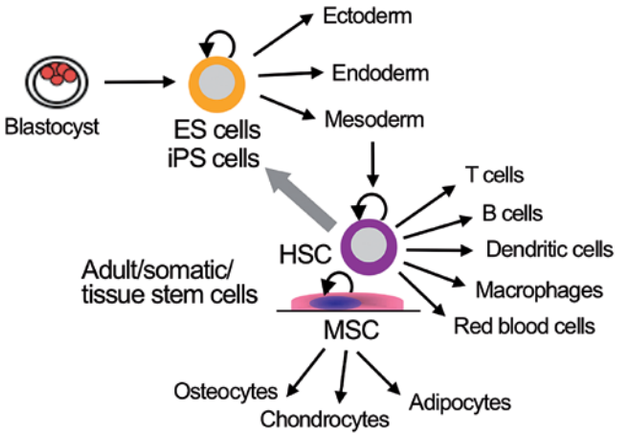

Fig. 1: Embryonic stem cells (ES cells) are pluripotent cells derived from the inner cell mass of blastocysts and give rise to cells of all three germ layers. Hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) develop into all cells of blood and connective tissue, respectively. Differentiated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

The Impact of TGF-β1 on Antigen Presenting Dendritic Cells

Transforming growth factor beta 1 (TGF-β1) is a pleiotropic cytokine involved in a variety of biological processes, such as development, differentiation, apoptosis and cell survival.

TGF-β1 is crucial for development of Langerhans cells (LC), the cutaneous contingent of DC. We have now investigated the influence of TGF-β1 on the bifurcation point of conventional DC (cDC) and plasmacytoid DC (pDC) by employing an *in vitro* culture system (Fig. 2). This *in vitro* system faithfully recapitulates DC development from hematopoietic stem cells, multipotent hematopoietic stem/progenitors (MPP) via Flt3$^+$M-CSFR$^+$c-kit^{low} common DC progenitors (CDP) into cDC and pDC (Figs. 2 and 3).

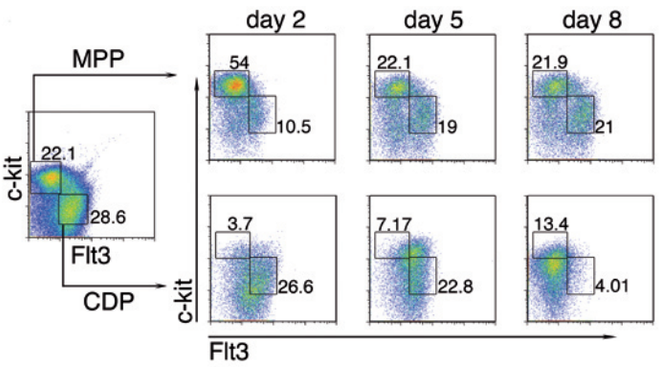

Fig. 3: MPP and CDP were FACS sorted, cultured under growth conditions (SCF, Flt3L, IGF-1, hyper-IL-6) and analysed by flow cytometry. MPP generate MPP and CDP, while CDP lack extensive self-renewal potential and differentiate into DC (Felker et al., 2010).

We found that TGF-β1 accelerates DC differentiation and directs differentiation towards cDC by inducing a cDC-affiliated transcription profile in CDP (Fig. 4).

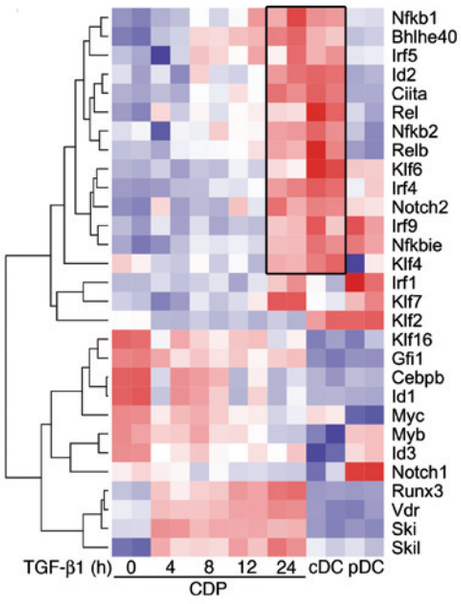

Fig. 4: TGF-β1 induces a cDC-affiliated transcriptional program in CDP, as revealed by transcriptional profiling and hierarchical cluster analysis. Data are shown in heatmap representation. Red, higher expression and blue, lower expression in relation to the mean expression value of the respective gene (Felker et al., 2010).

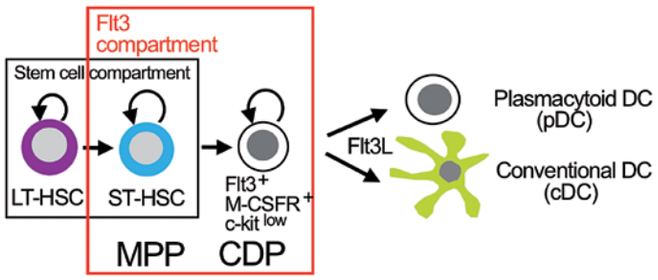

Fig. 2: Long-term reconstituting hematopoietic stem cells (LT-HSC) develop via MPP and CDP into pDC and cDC. Flt3L, Flt3 ligand.

Dendritic Cells Sense Biomaterials Through Toll-like Receptors

Biomaterials are used for a wide range of applications, including tissue regeneration, biosensors, joint implants and drug-delivery systems. Yet, biomaterials often cause inflammatory reactions, suggesting that they can profoundly alter the homeostasis of host immune cells. We have now analysed the influence of chemically and physically diverse biomaterials on DC using several mouse knockout models. DC express a large array of receptors, including Toll-like receptors (TLR), Fcγ receptors and C-type lectins, and recognise a myriad of antigens and "pathogen associated molecular patterns" (PAMP). We found that DC can sense biomedical polymers through a mechanism, which involves multiple TLR/MyD88-dependent signalling pathways, in particular TLR2, TLR4 and TLR6 (Shokouhi et al., 2010). Additionally, the engagement of TLR by biomaterials profoundly alters DC adhesive properties and induces prominent podosome formation (Fig. 5).

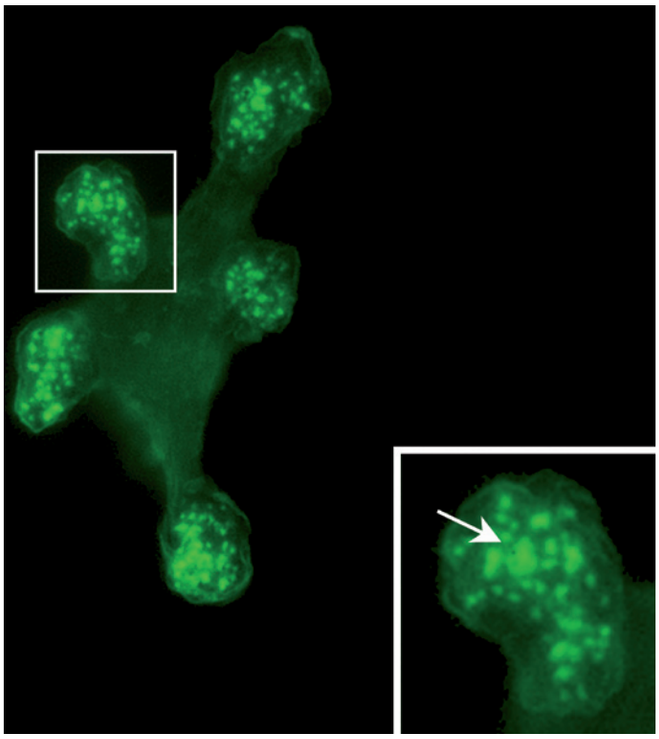

Fig. 5: Interaction of DC with the biomaterial polyethylene terephthalate (PET) results in large and clustered podosomes (Shokouhi et al., 2010).

Our findings should be useful for designing structure-function studies that aim at developing more bioinert materials. Moreover, they could also be exploited to generate biomaterials for studying the molecular mechanisms of TLR signalling and DC activation aiming at fine-tuning desired and pre-determined immune responses.

Reprogramming and Induction of Pluripotency

Pluripotency is defined as the capacity of cells to give rise to cell derivatives of all three germ layers (ectoderm, endoderm and mesoderm; Fig. 1). Recent progress in stem cell biology allows reprogramming and induction of pluripotency in somatic cells (referred to as induced pluripotent stem cells, iPS cells; Fig. 1) by expression of a defined set of transcription factors, including Oct4, Sox2, c-Myc and Klf4.

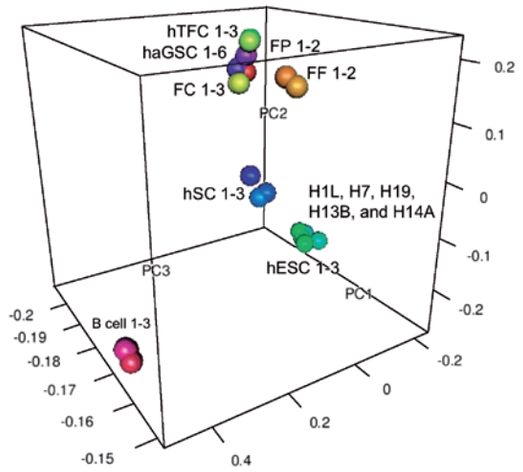

Fig. 6: Principal component analysis of genome-wide gene expression by DNA microarrays of human adult germline stem cells (haGSC), human ES cells (hESC) and human testicular fibroblast cells (hTFC). hSC, human spermatogonial cells; FF, foreskin fibroblasts; FC, normal colon fibroblasts; FP, parental foreskin cells (Ko et al., 2010).

Neural stem cells (NSC) endogenously express the two reprogramming factors Sox2 and c-Myc (Ruau et al., 2008; Kim et al., 2008) and this has allowed employing only two factors (Oct4 and Klf4) for obtaining iPS cells from NSC and eventually only Oct4 (Kim et al., 2008; 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; in collaboration with H. R. Schöler, MPI for Molecular Biomedicine, Münster, Germany).

Mouse gPS cells exhibit a gene expression repertoire, which is very similar to mouse ESC, as revealed by genome-wide gene expression profiling (Ko et al., 2009). In an analogous study we investigated gene array data of human adult germline stem cells (haGSC), which were claimed being pluripotent (Conrad et al., Nature 456, 344-349, 2008). We found that haGCS had a gene expression repertoire, which was similar to the expression repertoire of fibroblasts but different from the expression repertoire of human ESC (Ko et al., 2010; in collaboration with H. R. Schöler, MPI for Molecular Biomedicine, Münster, Germany). Thus, the pluripotency of haGSC is currently being questioned and a matter of debate. The study highlights the power of genome-wide gene expression profiling for quality control of pluripotent cells.

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 of differentiating into specific cell types and 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., 2010, 2011; Wein et al., 2010; Fig. 7).

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 (Cholewa et al., 2010, Horn et al., 2010, Wagner et al., 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; Koch et al., 2011; 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.

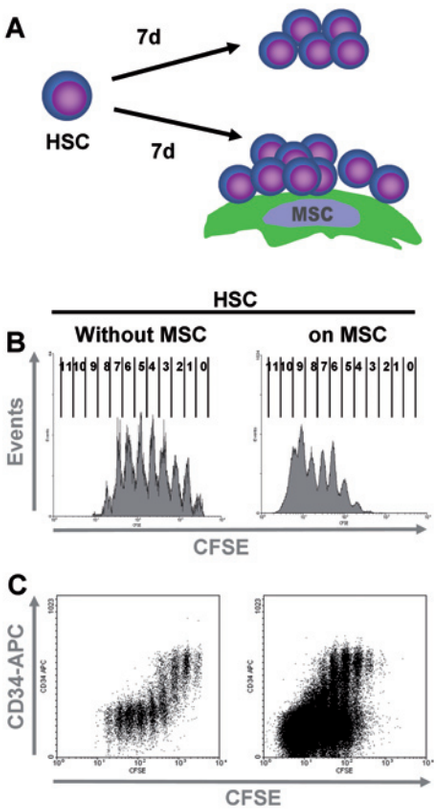

Fig. 7: Interaction of hematopoietic stem cells (HSC) with mesenchymal stromal cells (MSC). CD34⁺ cells from umbilical cord blood were cultured for 7 days with or without stromal support (A). The cell division history was analyzed by CFSE-fluorescent staining (B, the number of cell divisions is indicated). Proliferation is significantly enhanced by co-culture with MSC and they maintain CD34 expression for more cell divisions (C).

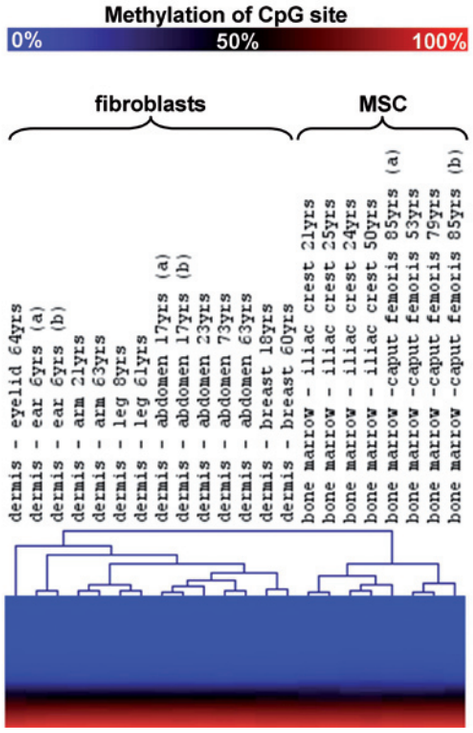

Fig. 8: Relationship of DNA methylation profiles. DNA methylation profiles of fibroblasts and MSC were analyzed with the HumanMethylation27 BeadChip that facilitates simultaneous analysis of 27,578 unique CpG sites. Unsupervised hierarchical clustering (Euclidian distance) of all CpG sites separated fibroblasts and MSC in two different groups. Notably, cells from the same anatomical site cluster together indicating that fibroblasts maintain a positional epigenetic memory (Koch et al., 2011).

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Team

Fig. 9: Stem Cell Biology and Cellular Engineering lab (03.11.2010).