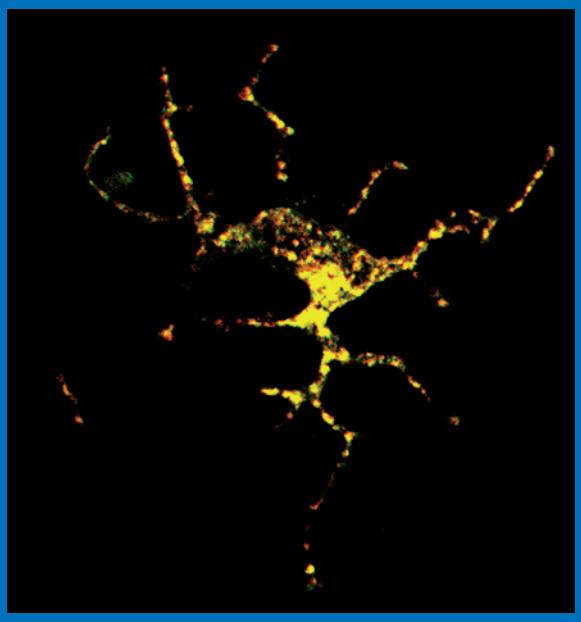
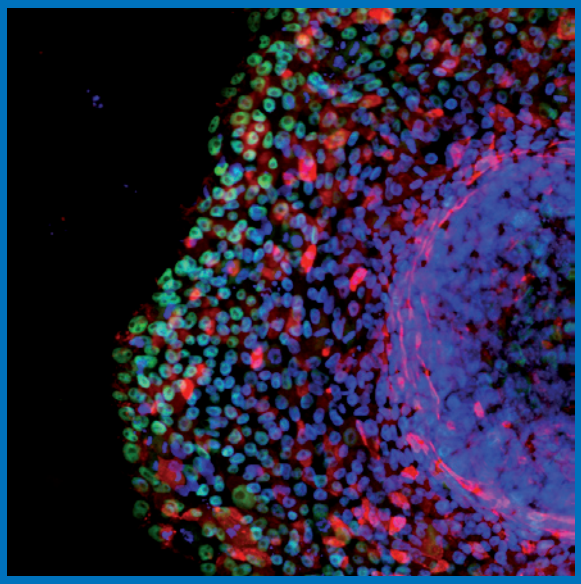
Gene Function in Cell Growth, Differentiation & Development





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Introduction

Engineering of stem cells and their differentiated progeny possesses enormous potential for biomedical applications and cellular therapies. It is well accepted that developmental routes and cell functions are determined by genetic programs. Thus, the laboratory studies the genetic and epigenetic regulation of hematopoietic stem cells (HSC), mesenchymal stem cells (MSC) and embryonic stem cells (ES cells) and the differentiation of specific lineages, such as antigen presenting dendritic cells (DC). In addition, reprogramming is used to enlarge the developmental options of somatic cells, referred to as induced pluripotent stem cells (iPS cells).

Stem cells and their differentiated progeny develop in a highly specialized microenvironment, referred to as stem cell niche. Therefore, a further objective of our research is to employ biomaterials and/or specific factors to recapitulate conditions of the stem cell niche *in vitro* for optimal growth and differentiation of stem/progenitor cells. This includes research on molecular mechanisms of cell-to-cell communication and cellular migration. To establish non-invasive tracking of cells *in vivo*, magnetic nanoparticles are employed as contrast agent in magnetic resonance imaging (MRI).

Since summer 2012 the institute hosts the newly recruited IZKF Research Group of Ivan Gesteira Costa Filho, PhD. The Costa groups aims at deciphering the molecular circuitries of cell differentiation, by employing bioinformatics tools and genome-wide data sets, including patient data.

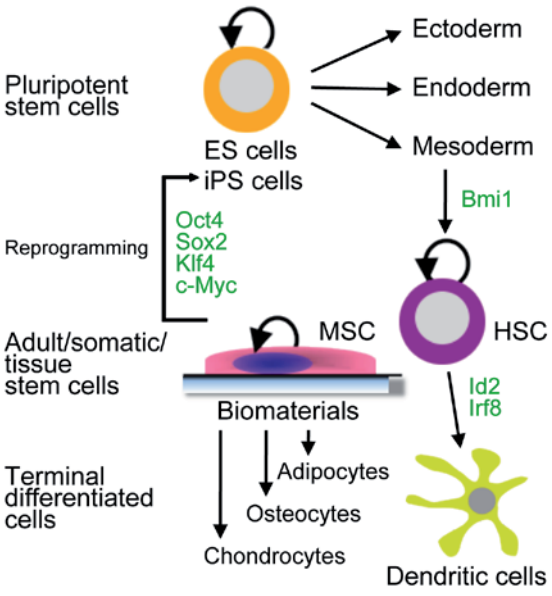

Fig. 1: Genetic programs determine cell fate. Transcriptional actuators (green) are investigated and used to influence developmental programs for cellular engineering. Hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) reside in the bone marrow niche and develop into all cells of blood, such as dendritic cells, and connective tissue cells, respectively. Biomaterials recapitulate aspects of the niche and impact on cell growth and differentiation. Induced pluripotent stem cells (iPS cells) are obtained from somatic cells by reprogramming.

Distinct Cytokine Signals Instruct Dendritic Cell Development and Function

DC are highly specialized immune cells with a unique role in antigen presentation and induction of primary immune responses. DC develop from hematopoietic stem cells guided by instructive cytokine signals, which are produced by the local microenvironment. DC development progresses from multipotent hematopoietic progenitors (MPP) via common DC progenitors (CDP) into conventional DC (cDC) and plasmacytoid DC (pDC) (Fig. 2).

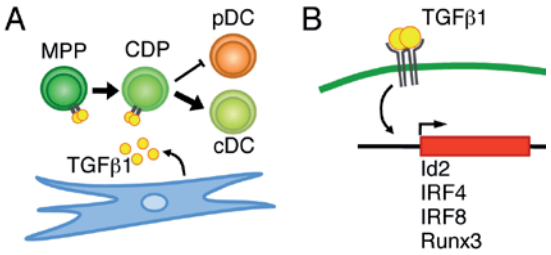

Fig. 2: (A) TGF-β1 produced by stroma cells impacts on DC lineage commitment and differentiation. (B) TGF-β1 induces expression of Id2, Irf8, Irf4, and Runx3, which are key transcription factors in DC development (Seré et al., 2012a).

We have investigated the activity of specific cytokines on DC subset specification and DC function, with a particular focus on TGF-β1 and hepatocyte growth factor (HGF) signalling (Seré et al., 2012a; 2012b; 2012c, Baek et al., 2012).

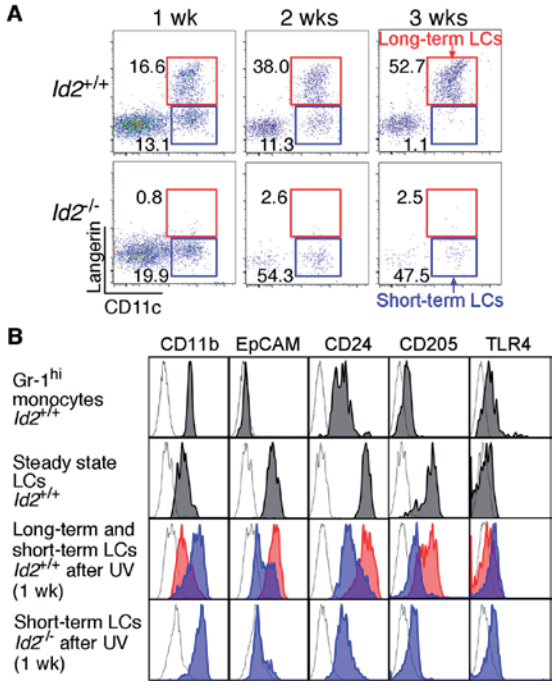

Fig. 3: Short-term and long-term LC differ in surface marker and gene expression (Seré et al., 2012b). (A) Id2+/+ and Id2-/- mice were exposed to UV light and cells from skin epidermis were analyzed by flow cytometry. (B) Surface marker expression on blood-derived Gr-1high monocytes and steady state LC and on cells from Id2+/+ and Id2-/- mice 1 week after UV exposure (red and blue histograms, long-term LCs and short-term LCs, respectively). Isotype controls, open histograms.

TGF-β1 impacts on the development of Langerhans cells (LC), the epidermal contingent of DC. We described two types of LC, long-term and short-term LC, which develop via separate pathways in steady state and inflammation, respectively. We propose that these two types of LC differ in their requirement for TGF-β1 (Seré et al., 2012b; 2012c).

We discovered that HGF signalling via the Met receptor tyrosine kinase provokes emigration of DC from skin and thus antagonizes the TGF-β1 effect. We described molecular mechanisms and the consequences of a lack of HGF/Met signalling in DC (Baek et al., 2012).

The StemCellFactory – Automated Derivation, Expansion and Differentiation of iPS Cells

Recent progress in reprogramming of adult cells towards pluripotency, referred to as iPS cells, introduced a new dimension to drug development and regenerative medicine, since it allows the generation of tissue-, disease- and patient-specific cells. To develop iPS cell production towards an industrial scale, the StemCellFactory consortium builds a fully automated production facility for patient-specific iPS cells and their neural and cardiac derivatives (Fig. 4). The facility comprises automation, standardization, and parallelization of all required cell culture steps, including reprogramming, up-scaling and a comprehensive quality management system. The StemCellFactory consortium combines leading forces in stem cell research and engineering sciences in North Rhine Westphalia, based in Aachen, Bonn, Leverkusen, Münster and Herzogenrath (www.stemcellfactory.de)

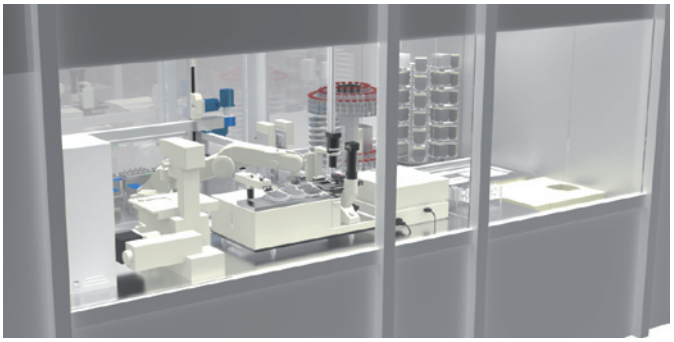

Fig. 4: View of the automated production facility for the generation of iPS cells and their neural and cardiac derivatives, referred to as StemCellFactory.

Rejuvenation by Reprogramming into iPS cells

Aging is a complex process, which affects every cell of the organism and leads to the deterioration of body functions over life time. Furthermore, cells undergo a process of "replicative senescence" during culture expansion. After a limited number of cell divisions, cells reflect changes in cellular morphology, proliferation and differentiation potential and ultimately enter growth arrest. We demonstrated that replicative senescence and cellular aging is associated with specific epigenetic modifications: specific DNA sequences become methylated and these modifications seem to be associated with the histone code (Fig 5). In fact, the state of cellular aging can be determined by analysis of DNA methylation of six genomic locations (Koch et al., 2012b; 2012c).

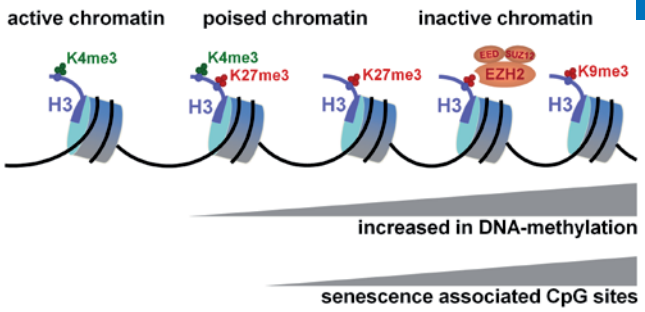

Fig. 5: Epigenetic modifications upon long-term culture of cells. Senescence-associated DNA methylation is preferentially acquired in genomic regions with repressive histone marks.

Notably, epigenetic modifications, which resemble cellular aging, are reversed upon reprogramming into iPS cells (Fig. 6). Our results support the notion that aging and replicative senescence are not only due to a random accumulation of cellular defects but are governed by a precisely controlled molecular program. Furthermore, cells can be rejuvenated by reprogramming into a pluripotent state.

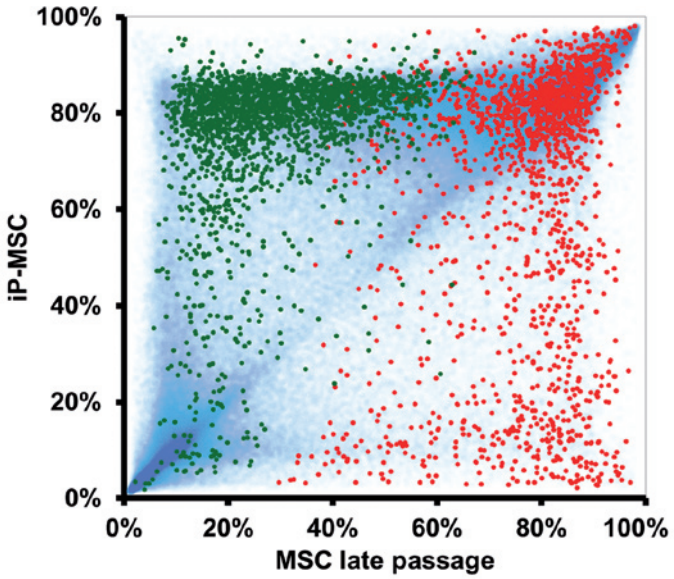

Fig. 6: Global DNA methylation profiles of MSC and induced pluripotent MSC (iP-MSC). The scatter plot represents DNA methylation at more than 480,000 genomic locations. Sites that become hypermethylated upon cellular aging are indicated in red, whereas hypomethylated sites are indicated in green. Reprogramming of MSC into iP-MSC specifically antagonized most of these senescence-associated modifications (Koch et al., 2012a).

Computational Biology of Cell Differentiation and Gene Regulation

Mechanisms, such as DNA methylation and histone modifications, remodel chromatin structure on a genome-wide scale. We use and develop bioinformatic approaches for integrated analysis of genome-wide gene expression data, histone modifications (chromatin immunoprecipitation sequencing, ChIP-Seq) and open chromatin assay (DNase). We developed a method based on sparse linear regression estimation, to predict the regulatory roles of transcription factors and histone modifications during blood cell development (Fig. 7; do Rego et al., 2012).

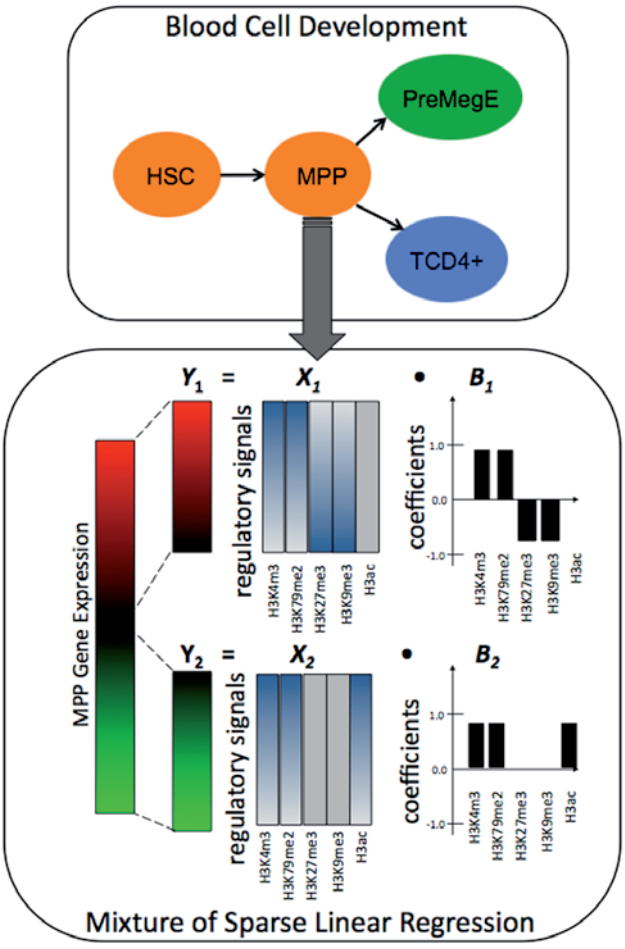

Fig. 7: Schematic blood cell development tree and a model inferred on MPP. The mixture model predicts the expression of genes of a particular cell type Y by the regulatory signals of the genes X. The coefficients B indicate the roles of each regulatory signal.

By using a hidden Markov Model based methodology the predictions for transcription factor binding sites were found to be more precise (Fig. 8; Gusmao et al., 2012).

A second line of research is the use of machine learning methods to ease the clinical diagnosis from patients' gene expression profiles. We performed a comprehensive study indicating characteristics of gene expression data sets, which reveals whether a computational diagnosis is difficult or not (Lorena et al., 2012). We also investigated the benefit of distinct proximity indices in clustering of patient expression profiles (Jaskowiak et al., 2012).

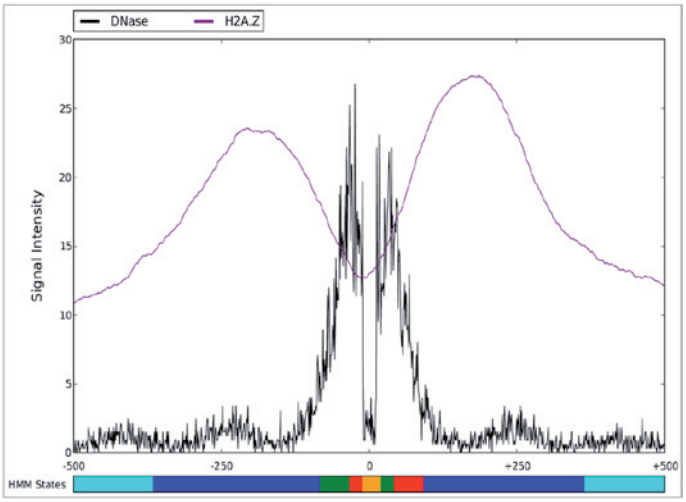

Fig. 8: The average profile of the activating histone H2AZ and the DNase sensitivity around CTCF binding sites (yellow position).

A third topic of research is the detection of structural variations from an individual patient with DNA sequencing technologies (DNA-Seq). In collaboration with CWI Amsterdam, The Netherlands, we have developed a graph-based algorithm and statistical framework for improving the accuracy of the prediction of medium sized structural variants (Marschall et al., 2012).

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Patent applications

Wagner W, Koch CM, Lin Q. Method for determining the biological age of a human donor (Epigenetic-Aging-Signature); 2012; EP12185698.3

Team

Above: Stem Cell Biology and Cellular Engineering lab
Right: Computational Biology Research Group