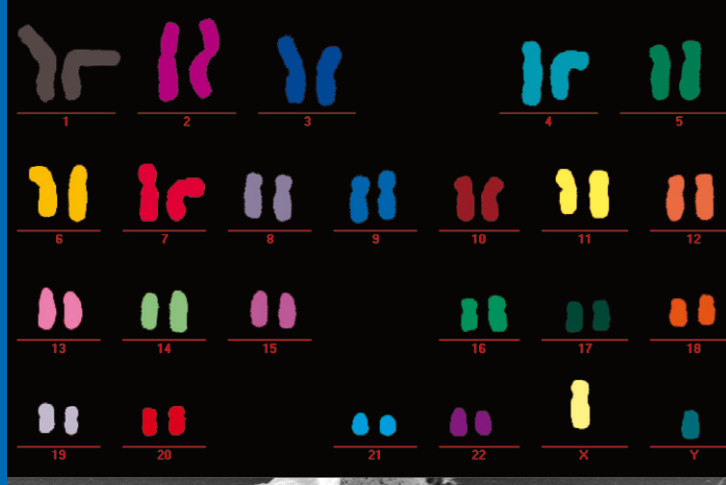
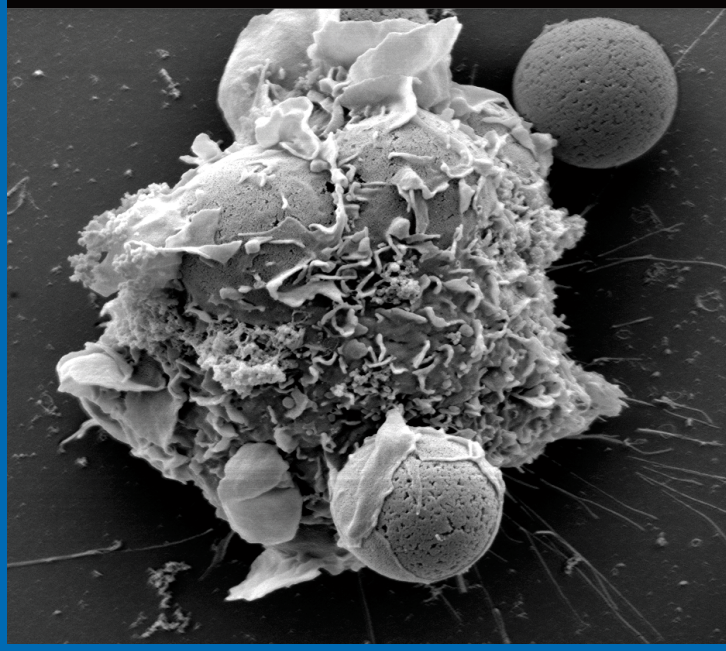
Gene Function in Cell Growth, Differentiation & Development





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Introduction

Signalling pathways impact on gene expression and determine cell identity and function (Fig. 1). The laboratory studies stem cells, including hematopoietic stem cells, embryonic stem cells (ES cells) and mesenchymal stem cells (MSC) and their differentiated progeny. Stem cells are unique in that they combine two properties in one cell: a high self-renewal activity and a broad multilineage differentiation potential. We employ (i) stem cell engineering to generate induced pluripotent stem cells (iPS cells) and (ii) genome precision engineering with CRISPR/Cas to generate cells with wanted properties. In addition, our studies build on a strong expertise in bioinformatics and computational biology for data analysis and prediction.

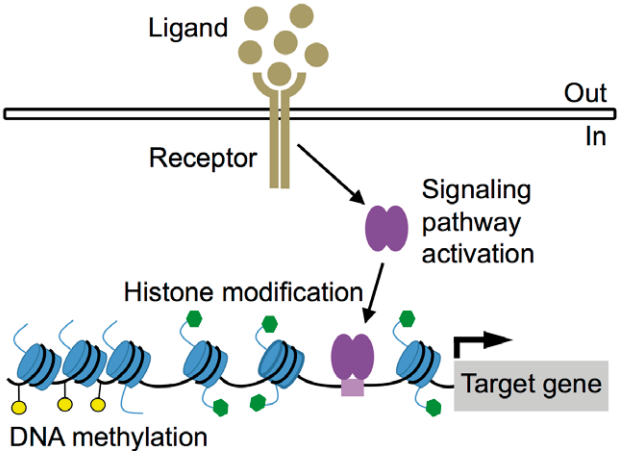

Fig. 1: Ligand binding to cognate receptor induces signalling pathways, transcription factor binding to DNA, chromatin modifications and target gene activation. Histone modification (green); DNA methylation (yellow).

Induced Pluripotent Stem Cells

Pluripotent stem cells, including ES cells and iPS cells, provide unique opportunities for disease modelling, drug development and cell therapy. However, frequently their differentiation potential is rather poor, in particular towards mesodermal lineages, such as hematopoietic cells. We used cell fusion of ES cells (or iPS cells) with hematopoietic stem cells to increase the propensity and differentiation potential of pluripotent stem cells towards mesodermal lineages (Qin et al., 2014; Fig. 2).

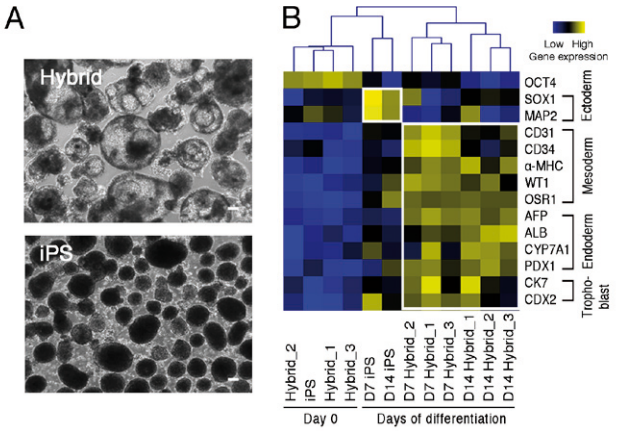

Fig. 2: (A) Human iPS cells fused with hematopoietic stem cells (Hybrid) show prominent cystic structures in EB assays, indicating differentiation bias towards mesoderm. (B) Gene expression profiling demonstrates mesendodermal differentiation bias of hybrids.

iPS cells represent a particularly appealing cell source for personalized regenerative therapies, since autologous iPS cell-derived cells are expected to bypass immune rejection. However, this assumption has remained controversial. We generated iPS cells from immune-privileged Sertoli cells of testis (Ser-iPS cells; Wang et al., 2014). Ser-iPS cells were less immunogenic in vivo and in vitro than iPS cells obtained from mouse embryonic fibroblasts (MEF-iPS cells). Ser-iPS cells exhibited an immunogenicity similar to isogenic ES cells (Wang et al., 2014; Fig. 3). Our data suggest that immune-privileged Sertoli cells might represent a preferred source for iPS cell generation if it comes to the use of iPS cell-derived cells for transplantation.

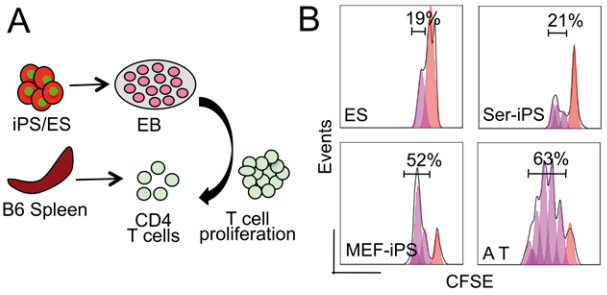

Fig. 3: Ser-iPS cells exhibit reduced CD4 T cell stimulation potential in vitro. MEF-iPS cells show some immunogenicity and T cell stimulation potential.

The *StemCellFactory* consortium (www.stemcellfactory.de) is currently testing and further developing the prototype of an automatic production facility for patient-specific iPS cells (Fig. 4). The *StemCellFactory* consortium combines leading experts in stem cell research and engineering sciences in North Rhine Westphalia, based in Aachen, Bonn, Münster and Herzogenrath.

Fig. 4: StemCellFactory, an automatic production facility for patient-specific iPS cells.

Magnetic Nanoparticle-labelling of Cells and Tracking by MRI

Labelling of cells with engineered magnetic nanoparticles (MNP) before implantation shows great promise in monitoring successful cell deposition, differentiation, and migration using magnetic resonance imaging (MRI). One obstacle is to achieve a stable long-term labelling of stem and progenitor cells with MNP. One approach for tailoring of MNP properties is the Layer-by-Layer (LbL) assembly of polyelectrolytes (PE) around iron-oxide cores (in collaboration with J. E. Wong, Chemical Process Engineering, AVT.CVT, Faculty of Mechanical Engineering, RWTH Aachen University, Aachen, Germany). We recently investigated PE-coating of ferumoxytol, which is an FDA and EMA approved drug (Celikkin et al., 2014, Fig. 5). We found that the molecular weight of PE is a critical parameter to shape particle size and structure of ferumoxytol MNP. Importantly, the labelling efficiency was significantly higher when PE-coated ferumoxytol particles were used for labelling of mouse bone marrow derived hematopoietic stem cells and dendritic cells (DC) (Celikkin et al., 2014, Fig. 5). Further attempts aim at endowing MRI contrast agents with additional functionalities. Thus, we currently focus on the use of fluorescently labelled PE for coating of MNP using LbL assembly to generate bimodal contrast agents that are suitable for both optical and MR imaging.

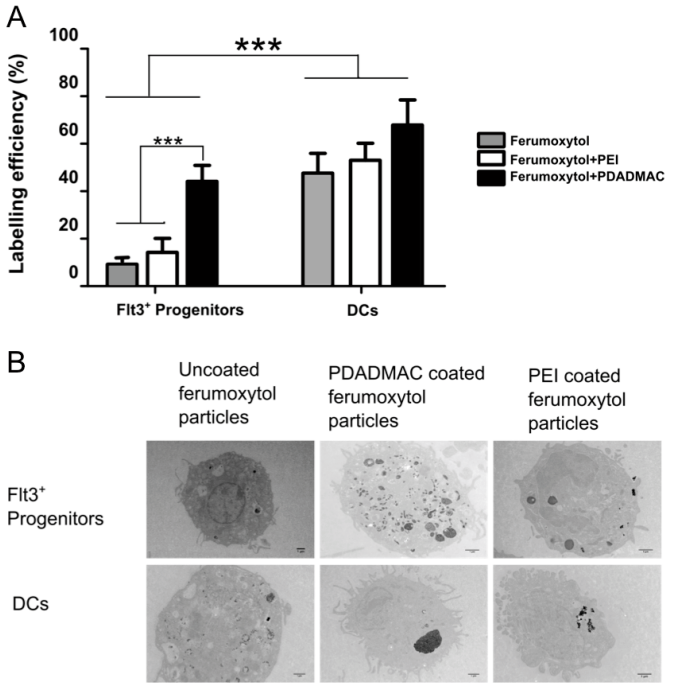

*Fig. 5: Labelling of cells with PE-coated and uncoated ferumoxytol particles. (A) Results of labelling efficiency are mean values ± SD (n=3; ***: p<0.01). (B) Transmission electron micrographs of MNP-labelled cells. Scale bars, 1 µm.*

Regulation of Actin Cytoskeleton Dynamics and Cell Motility

Remodelling of the actin cytoskeleton is fundamental for many biological processes including cell motility, embryonic development and the immune response. In the context of the immune response, Fcγ receptor-mediated phagocytosis by macrophages and DC plays a crucial role for efficient pathogen recognition and clearance. Fcγ receptor-mediated phagocytosis depends on actin cytoskeleton remodelling, but the molecular basis underlying this process is still incompletely understood. We have found that the leukocyte-specific protein 1 (LSP1) co-localises with actin to nascent phagocytic cups during Fcγ receptor-mediated phagocytosis (Fig. 6). Down regulation of LSP1 severely impaired Fcγ receptor-mediated phagocytosis. Moreover, LSP1 binds to the class I molecular motor myosin1e. The inhibition of LSP1-myosin1e interaction greatly impairs pseudopodia formation around opsonised targets and their subsequent internalisation. Hence, our findings indicate that LSP1-myosin1e bi-molecular complex plays a crucial role in the regulation of actin cytoskeleton remodelling during Fcγ receptor-driven phagocytosis (Maxeiner et al., in revision, Fig. 6).

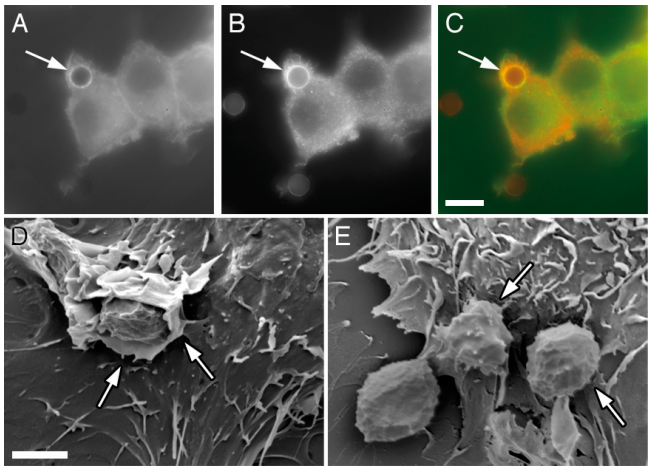

Fig. 6: (A-C) Co-localisation of actin (A) and LSP1 (B) during Fcγ receptor-mediated phagocytosis. Arrows indicate accumulation of actin and LSP1 around opsonised beads. Scale bar: 10 µm. (D-E) LSP1 down regulation impairs lamellipodia formation around opsonised beads. In control cells (arrows in D), opsonised red blood cells (RBC) are surrounded by lamellipodia during Fcγ receptor-mediated phagocytosis. By contrast, in LSP1-deficient cells lamellipodia formation at RBC-cell contact sites is inhibited (arrows in E). Scale bar: 5 µm.

Epigenetic Rejuvenation of iPS-derived Mesenchymal Stem Cells (iPS-MSC)

MSC comprise a multipotent cell population able of differentiating into adipocytes, chondrocytes, and osteocytes. MSC raise high hopes for clinical application. However, primary cultures of MSC are heterogeneous and greatly

affected by the starting material, culture and isolation procedures. To overcome these obstacles we differentiated MSC from iPS cells by using a simple technique of switching to initial MSC-culture conditions (Frobel et al., 2014, Fig. 7).

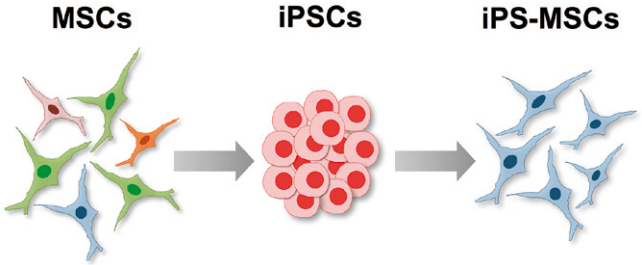

Fig. 7: Reprogramming of heterogeneous donor MSC into iPS cells and further differentiation toward standardized iPS-MSC.

Generated iPS-MSC showed the same morphology, immunophenotype and functional properties as parental MSC. Furthermore, gene expression profiles of iPS-MSC highly resembled those of MSC. By using our recently developed "Epigenetic-Aging-Signature" (Weidner et al., 2014), based on DNA methylation changes at specific CpG sites upon aging, we showed that iPS-MSC are estimated much younger than MSC. This demonstrates that the epigenetic rejuvenation upon reprogramming into iPS cells is also maintained in iPS-MSC (Fig. 8).

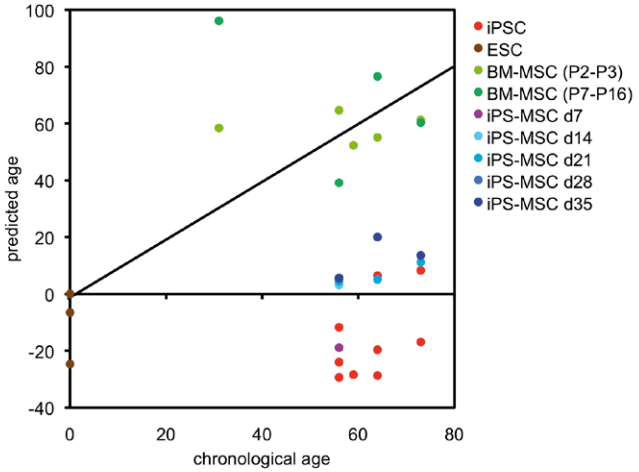

Fig. 8: iPS-MSC appear epigenetically rejuvenated when predicting the age with our recently developed "Epigenetic-Aging-Signature" based on the DNA methylation status of 99 specific CpG sites.

Besides aging, pluripotency is also associated with epigenetic changes. To better track the pluripotent state of cell preparations – for example during differentiation processes – we developed a tool based on DNA methylation changes at three specific CpG sites, called "Epi-Pluri-Score" (manuscript in revision; patent pending).

Computational Biology of Cell Differentiation, Diseases and Gene Regulation

Mechanisms, such as DNA methylation and histone modifications, remodel chromatin structure and regulate gene expression during cell differentiation and disease. Our main aim is the development of bioinformatics approaches for the integrated analysis of genome-wide gene data, such as gene expression, DNA methylation and histone modifications, to improve our understanding of these biological processes under normal and diseased conditions.
We have developed the first integrated method for the identification of changes in protein-DNA interactions in pairs of cellular conditions. The algorithm performs signal normalization, detection of differential peaks and *p*-value estimation in an integrative manner (Allhoff et al., 2014). An empirical analysis based on comparing gene expression with differential peaks from cell differentiation and response to treatments demonstrates that our differential peak predictions outperform most competing methods (Fig. 9).

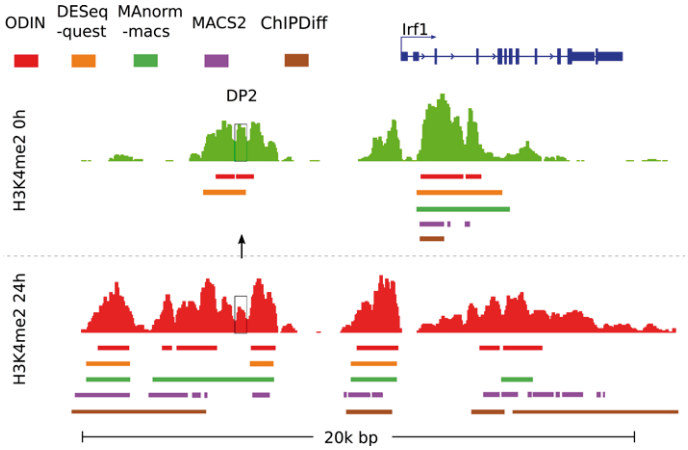

Fig. 9: Example of differential peaks detected by comparing H3K4me2 on 0h and 24h after TLR4 induction around the Irf1 gene.

We have also developed statistical tests and graphics methods to detect associations between regulatory regions (DNA-protein interaction sites) or between regulatory regions and genomic signals (Fig. 10). Test results and plots are presented in an html interface allowing a simple analysis of large amounts of genomic data. All the above-mentioned tools are implemented in the Regulatory Genomics Toolbox available at www.regulatorygenomics.org.

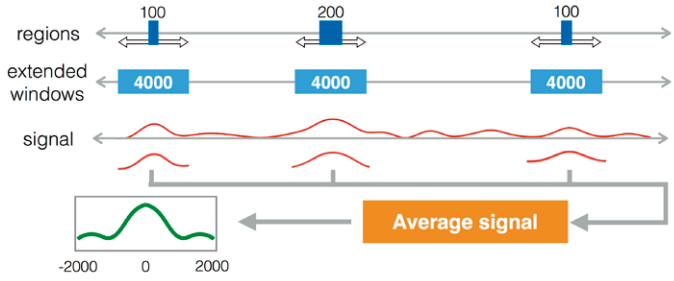

Fig. 10: Strategy for identifying spacial association between genomic regions and genomic profiles.

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Patent applications

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