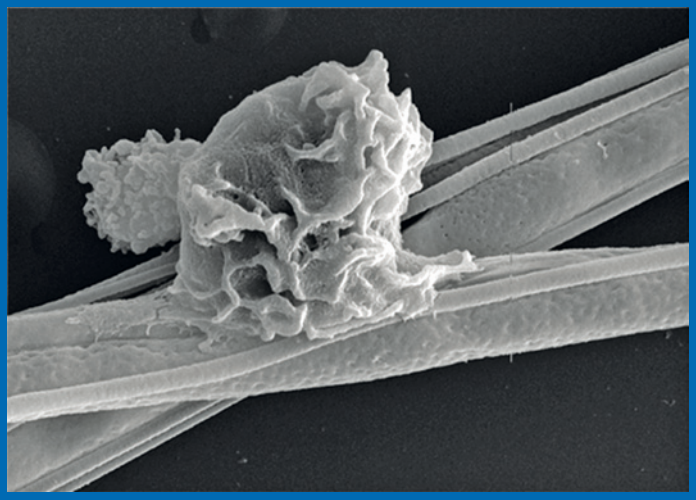
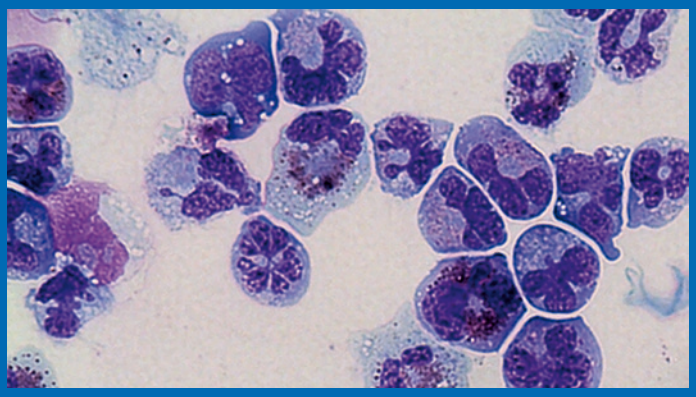
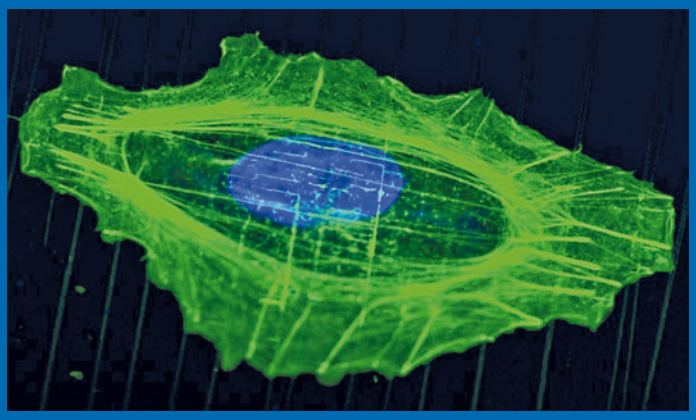
Gene Function in Cell Growth, Differentiation & Development







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Introduction

In cells a specific grammar of DNA and chromatin modifications determines gene expression and thus cell identity and function. We aim at elucidating how gene networks direct cell fate and specific cellular activities, using a rich toolbox of bioinformatics and computation for analysis and predictions of sequencing data (Fig. 1). The laboratory studies stem cells both in the normal physiological and in the diseased state. This includes blood stem cells (hematopoietic stem cells, HSC), mesenchymal stem cells (MSC), embryonic stem cells (ES cells) and also engineered stem cells, such as induced pluripotent stem cells (iPS cells), and their differentiated progeny. We use genome precision engineering with CRISPR/Cas to generate cells with desired properties. A particular focus is on antigen presenting dendritic cells (DC). We also study stem cell aging, magnetic nanoparticles for cell tracking and the influence of biomaterials and surface topology on cell growth and cell behavior.

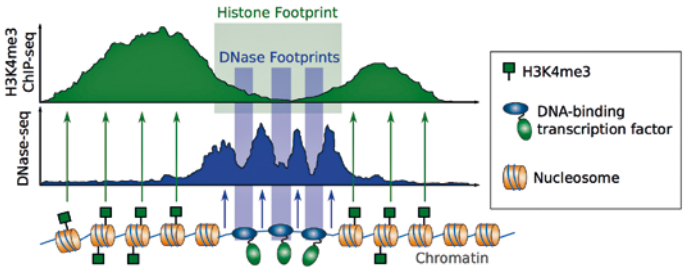

Fig. 1: The histone modification H3K4me3 is indicative of active genes. The binding of transcription factors to the promoters of these genes leaves histone and DNase footprints.

Engineered IRF8-/- iPS Cells by CRISPR/Cas Genome Editing

Human iPS cells can differentiate into cells of all three germ layers, including hematopoietic stem cells and their progeny. Interferon regulatory factor 8 (IRF8) is a transcription factor, which acts in hematopoiesis as lineage determining factor and autosomal recessive or dominant IRF8 mutations in patients cause severe monocytic and DC immunodeficiency.

To study IRF8 in human hematopoiesis we generated human IRF8–/– iPS cells and IRF8–/– ES cells using RNA guided CRISPR/Cas9n genome editing (Sontag et al., 2016, in press). We differentiated iPS cells and ES cells into hematopoietic stem/progenitor cells and further into DC. IRF8 deficiency caused a bias towards granulocytes at the expense of monocytes and compromised development of specific DC subsets (Fig. 2). Additionally, IRF8–/– DC showed reduced MHC class II expression and were impaired in cytokine responses, migration and antigen presentation. Thus, this human IRF8 knockout model allows studying molecular mechanisms of immunodeficiencies in the human system.

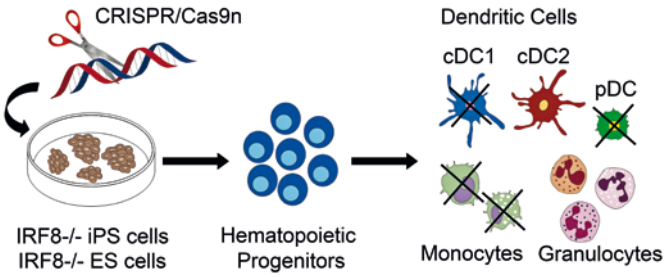

Fig. 2: IRF8–/– iPS cells and IRF8–/– ES cells were generated by CRISPR/Cas9n and differentiated into hematopoietic progenitors and further into mature blood cells. Deletion of IRF8 compromised development of specific dendritic cell subsets (classical DC1 and plasmacytoid DC, cDC1 and pDC, respectively) and monocytes, while enhancing the frequency of granulocytes (Sontag et al., 2016, in press).

Senescence-associated Epigenetic Modifications of Cells in Culture

MSC represent the cell type that is currently used in most clinical trials – but this necessitates *in vitro* culture expansion to achieve clinical relevant cell numbers. During the *in vitro* expansion, MSC acquire large and flat morphology, lose differentiation potential, and ultimately enter proliferation arrest – a state defined as replicative senescence. Interestingly, replicative senescence is reflected by continuous and highly reproducible DNA methylation (DNAm) changes at specific CpG dinucleotides in the genome. We developed a deep sequencing method based on six CpGs to precisely reflect the state of replicative senescence in MSC and human umbilical vein endothelial cells (HUVEC) (Fig. 3; Franzen et al., 2016; patent pending).

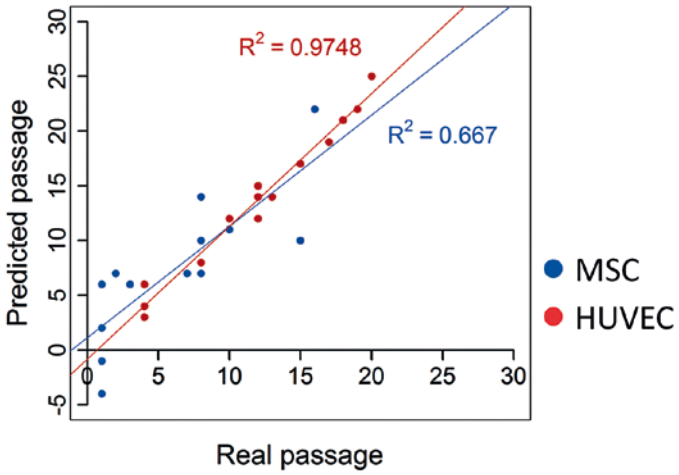

Fig. 3: Epigenetic-Senescence-Signature based on six CpG dinucleotides to predict the number of passages of cell preparations of MSC and HUVEC (Franzen et al., 2016).

It is still unclear whether the senescence-associated DNAm changes are directly regulated by a targeted molecular process. During the last years long non-coding RNA (lncRNA; >200 nucleotides) have emerged as

potential epigenetic modifiers. We demonstrated that the HOX transcript antisense RNA (*HOTAIR*) binds preferentially to genomic *loci* that become hypermethylated during replicative senescence. Gain- and loss-of-function approaches indicated that *HOTAIR* expression contributes to regulation of cellular senescence by changes in gene expression and DNAm. Notably, *in silico* and subsequent *in vitro* analysis indicated that targeting of *HOTAIR* to specific genomic *loci* is mediated by triple helix formation (Fig. 4; Kalwa et al., 2016).

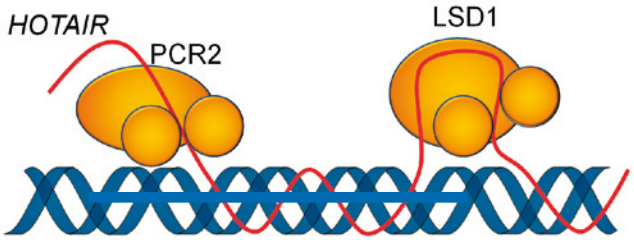

Fig. 4: Schematic representation of the triple helix formation. The lncRNA HOTAIR targets specific genomic locations and interacts with epigenetic modifiers, such as PCR2 and LSD1 (Kalwa et al., 2016).

Polyelectrolyte Coating of Magnetic Nanoparticles for Cell Labelling and Tracking by MRI

Translation of cell-based therapies into clinical applications is hampered due to the lack of tools to monitor cell fate and function after transplantation. Labelling of cells with engineered magnetic nanoparticles (MNP) before implantation shows great promise in tracking cells *in vivo* using magnetic resonance imaging (MRI).

We study the tailoring of MNP using layer-by-layer assembly of polyelectrolytes (PE) for enhanced labelling of hematopoietic stem/progenitor cells and DC and their tracking using MRI (Schwarz et al., Nanomedicine, 2012; Celikkin et al., J. Magn. Magn. Mater, 2015; in collaboration with W. Richtering and J. E. Wong, Institute of Physical Chemistry, RWTH Aachen University, Aachen, Germany and M. Hoehn, In vivo NMR Research Group, MPI for Metabolism Research, Cologne, Germany). Recent studies revealed a differential impact of specific PE coatings on labelling and cellular responses of different DC subsets under steady state and inflammatory conditions (Celikkin et al., manuscript in preparation; Fig. 5).

Regulation of Cell Motility and Adhesion by Surface-grafted Nanogel Arrays

Cellular functions, including cell adhesion and migration, are controlled by the precise spatio-temporal regulation of cytoskeleton dynamics. Several interdisciplinary studies have demonstrated that material chemistry and topology can be exploited to modulate cell adhesion and migration.

In this project, we developed highly functional and stimuli-responsive poly(*N*-isopropyl acrylamide) nanogel arrays grafted onto glass surfaces by a printing process using wrinkled polydimethylsiloxane (PDMS) templates (Sechi et al., 2016). Using low-temperature plasma treatment, nanogels were chemically grafted onto glass supports thus leading to highly stable nanogel layers in cell culture media.

We demonstrate that surface-grafted nanogels can serve as novel substrates for the analysis of cell adhesion and migration (Sechi et al., 2016). Nanogels have a strong impact on size, speed and dynamics of focal adhesions and cell motility causing cells to move along straight trajectories (Fig. 6). In addition, modulation of nanogel swelling state or spacing serves as an effective tool for regulation of cell motility.

Our study demonstrates that nanogel arrays deposited on solid surfaces can be used to provide a precise and tunable system to understand and control cell migration. We anticipate that our surface-grafted nanogel system will contribute to the development of implantable systems aimed at supporting and enhancing cell migration and adhesion.

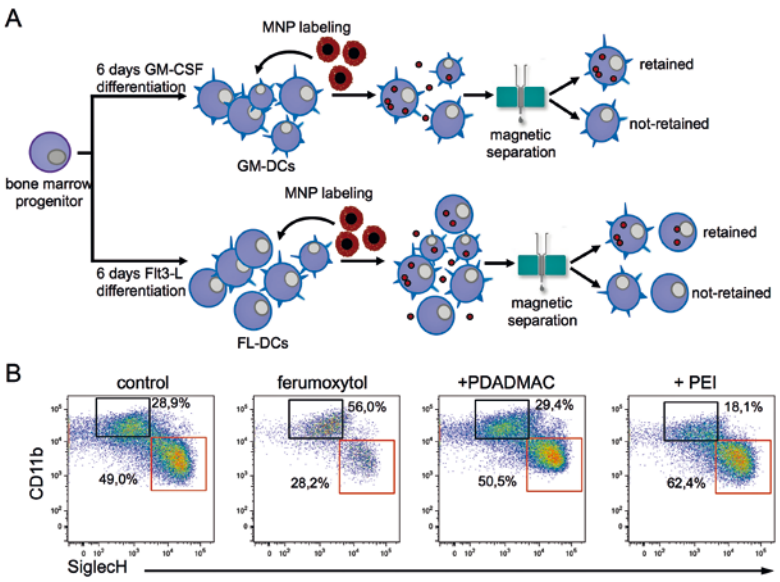

Fig. 5: Labeling of inflammatory (GM-DC) and steady state (FL-DC) DC with PE-coated and uncoated ferumoxytol particles. (A) Schematic representation of magnetic separation after labelling with MNP. (B) FL-DC after uptake of respective MNP were analyzed by flow cytometry. Representative dot plots show MNP-labeled cDC (black boxes) and pDC (red boxes) subsets.

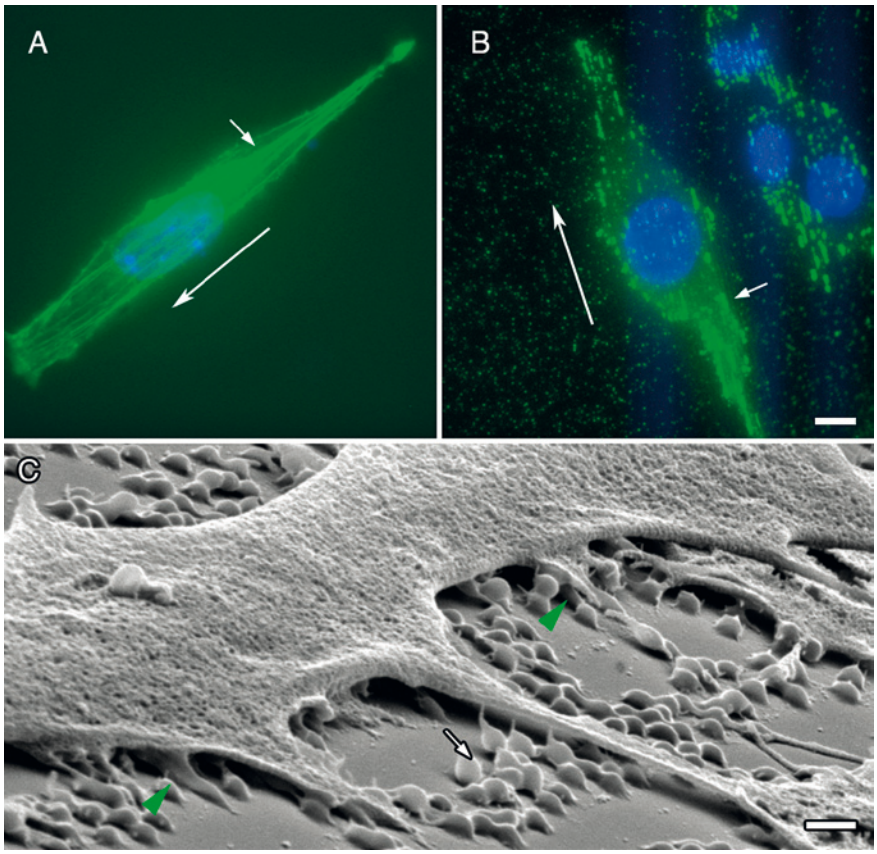

Fig. 6: Impact of surface-grafted nanogels on cell morphology and cytoskeleton organisation (A-C). The microfilaments (A, green, arrow) and focal adhesions (B, green, arrow) of mouse melanoma B16F1 cells aligned along the major axis of nanogel arrays (long arrows). Cell nuclei were stained with DAPI (blue). Scale bar: 10 µm. Scanning electron microscopy of B16F1 cells seeded on nanogel arrays (C). Note the interaction of cell projections (green arrows) with the underlying nanogel arrays (white arrow). Scale bar: 0.25 µm (Sechi et al., 2016).

Computational Detection of Cell Specific Binding Sites

One of the main molecular mechanisms controlling the temporal and spatial expression of genes is transcriptional regulation. In this process, transcription factors bind to the vicinity of a gene to recruit (or block) the transcriptional machinery. The identification of transcription factor binding sites is a first step to understand regulatory networks driving cellular processes, such as cell differentiation and the onset of diseases. Cell specific binding sites can be predicted by the analysis of sequencing protocols measuring open chromatin with computational footprinting methods (Fig. 7).

We have revisited the problem of binding site detection from computational footprinting in Gusmao *et al.*, 2016. We showed that several state-of-the-art methods (including our own method) can detect cell specific binding sites with very high accuracy. Moreover, we demonstrated how to correct for experimental artefacts, such as DNase I enzyme cleavage bias, thereby settling a long-standing scientific discussion about computational footprinting (see also Editorial Nat. Methods 13, 185, 2016). In practical examples, we have applied computational footprint to dissected NF-kB regulation during inflammation in HUVEC (Kolovos et al., 2016) and to uncover regulatory roles of Tbx3 in pancreas development (Perkhofer et al., 2016).

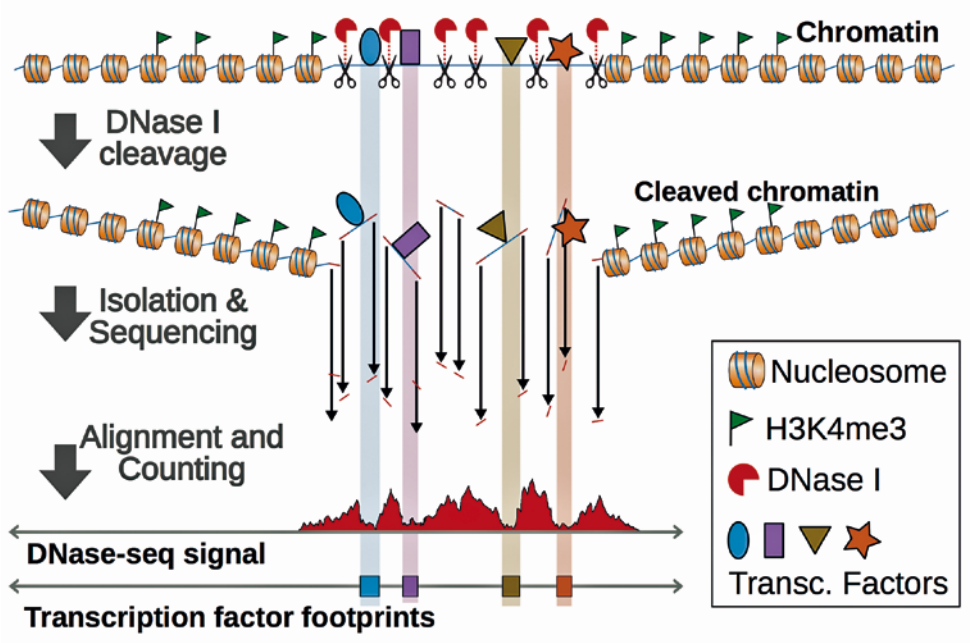

Fig. 7: Workflow of the computational analysis of the open chromatin protocol DNAse-seq (Gusmao et al., 2016).

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Patent applications

[1] Epigenetic classification of human mesenchymal stromal cells; 2016; EP 16152198.4 (Wagner W, de Almeida DC)

[2] Method for analysis of the cellular composition in buccal swabs; 2016; DE 10 2016 109 291.6 (Wagner W, Eipel M)

Team

At the Top and right: Best Paper Award to Eduardo Gusmao, PhD (right) and Poster Award to Thomas Hieronymus, PhD (top, second from left) at Medical Sciences Day 2016, Medical Faculty RWTH Aachen University.

Below: Lab retreat at Mosel river.